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Contents

Social evolution, queen number and supergenes

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Social insects exhibit spectacular intraspecific polymorphism, from individual castes to variation in social organization among social groups. Insect societies therefore provide great opportunities to study how the genome and environment interact to produce phenotypic diversity. In this lecture, I will focus on the genetic underpinnings of alternative forms of social organization in ants and discuss the role of supergenes in controlling complex phenotypes.

Socially polymorphic ant species have two types of colonies, headed by a single queen or by multiple queens, respectively. The two social forms differ in many morphological, behavioural and life-history traits, and this "polygyny syndrome" has arisen independently in multiple ant lineages. Hence, by comparing the mechanisms underlying convergent variation in social organization, we can better understand how genomic evolution contributes to phenotypic innovations.

In the Alpine silver ant *Formica selysi* and the fire ant *Solenopsis invicta*, colony social organization is controlled by supergenes, which are clusters of tightly linked genes. These supergenes have evolved independently in these two distant lineages, and they show no detectable similarity in gene content. In both systems, one of the two haplotypes of the supergene is preferentially transmitted, yet the mechanisms of this transmission ratio distortion differ. Thus, the unusual behaviour of the underlying genetic system contributes to the maintenance of the social polymorphism.

More generally, the evolution of supergenes, through the suppression of recombination, preserves beneficial associations of alleles across co-adapted traits. Supergenes control complex polymorphic phenotypes, including sexes, ecotypes, cryptic morphs, or divergent social phenotype in insects and birds, and thus play an important role in social evolution

The breakdown of cooperation in natural populations of bacteria and birds

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A typical way to approach the challenge of understanding social behaviours is to start by imagining a population of selfish individuals and thinking about how they might evolve to start cooperating with one another. In this talk, I will present work on two very different systems where we observe this evolutionary process in reverse. In the first case, we have used a combination of experiment and genome analyses to understand why bacterial cells gradually become more selfish in the way that they scavenge iron in the human lung. In the second, we have reconstructed the ancestral state of birds and used comparative analyses to understand why some cooperative breeders lose their helpers. In both cases, we were able to identify selection pressures responsible for the breakdown of cooperation but these were not always as expected from our understanding of how they may have evolved to cooperate in the first place.

Social insects and the evolution of social learning

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Across the animal kingdom, from primate troops to bird flocks, fish shoals to social insect colonies, individuals respond to information provided by others. Yet we still understand little of how natural selection has shaped social learning abilities, or indeed whether it has shaped them at all. Is social learning a cognitively distinct process from individual learning? Here, I will demonstrate how social insect models can be used to investigate the mechanistic basis for social learning phenomena. These studies illustrate how apparently sophisticated social learning can often be explained by simple associative processes that have little to do with sociality. Insects are changing the way that we think about the cognitive requirements for social life, because an invertebrate perspective encourages close scrutiny of apparently complex cognitive phenomena.

Cardiocondyla coming of age

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Cardiocondyla ants have small colonies, a short generation time, and they can be bred easily in the lab. They thus in an almost unique way combine traits that make them ideal models for investigations on life span and life time reproductive success. Our previous studies have shown that in the laboratory the fecundity of queens increases with age and that life span and weekly egg laying rate are positively associated.
Building up on the recent sequencing of the genome of *C. obscurior* we have started to analyze how gene expression changes with individual condition and age. We found that the expression of many genes changes with age in an opposite way in *C. obscurior* queens than in Drosophila. For example, genes related to reproduction were upregulated in older queens, while genes associated with metabolic processes and muscle contraction were downregulated. This matches the absence of a profound reproductive senescence in *C. obscurior* queens. While increased reproductive effort did not result in shortened lifespan, an injury-induced immune response temporarily decreased egg laying rate. This indicates that, while it is difficult to substantiate a trade-off between fecundity and longevity under normal laboratory conditions with worker care and ad libitum food, challenging an individual queen's condition can reveal hidden costs of reproduction that are normally borne by workers.

Remoulding of the fecundity/longevity trade-off in a fungus-growing termite

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Ageing occurs in nearly all organisms and it appears to trade off with fecundity. In some organisms, such as queens of social insects, this trade-off is apparently absent, allowing some individuals to live long and to have a high reproductive rate. An extreme example is the mound building [West African] termite *Macrotermes bellicosus*. Here queens (and kings) can live for 20 years and at the same time lay 20.000 eggs per day while workers live only a few weeks. We aim to shed light on the connection between fecundity, longevity and sociality in termites by comparing transcriptomes of young and old queens, kings and two sterile worker castes.

A principal component (PC) analysis separated our samples according to caste and age. Along the first PC, queens, kings and workers were distinct, but major and minor workers clustered together. Along the second PC, expression differences were most pronounced between young and old major workers and to a somewhat lesser extent in small workers. By contrast, both sexual castes, were not spread along the second PC although individuals differed in age by 10 years and more. This is consistent with the hypothesis that reproductives do age more slowly. We will discuss genes loading on this axis and compare them with genes associated with ageing in social Hymenoptera and solitary insects. This will allow us to obtain insights into common underpinnings of the fecundity/longevity trade-off in social insects.

The co-regulation of fertility and the mode of parthenogenesis in parasitic workers of the honeybee (*Apis mellifera capensis*)

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In honeybees worker reproduction is rare in the presence of a queen. Only if the queen gets lost and there is no brood in the colony workers can activate their ovaries and lay unfertilized haploid eggs that develop into males (= arrhenotokous parthenogenesis). An exception are laying workers of the South African Cape bee *Apis mellifera capensis.* Workers of this subspecies are able to produce female progeny via thelytokous parthenogenesis. Thelytoky as well as some other phenotypic traits providing Cape bees with reproductive dominance (high number of ovarioles, early onset of egg-laying and production of a queenlike mandibular gland pheromone) have been suggested to be controlled by a recessive allele at the *thelytoky* locus (*th*). We here report on experiments free of intracolonial selection for reproductive dominance finding the mode of parthenogenesis in laying *A. m. capensis* workers to be dimorphic, segregating in 50% thelytokous and 50% arrhenotokous workers. This Mendelian segregation suggests the queen to be heterozygous at the *thelytoky* locus with all siring males carrying the *th* allele and therefore a single locus controlling thelytoky.

Reproductive traits of females and males in two invasive hornets.

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Vespids are very efficient invaders, especially thanks to the plasticity that their sociality provides. This is the case in two hornet species, the European hornet invasive in America, *Vespa crabro*, and the Chinese hornet invasive in Europe since 2004, *V. velutina*. *V. crabro* makes middle sizes colonies and has an essential position in the European ecosystem, while *V. velutina* makes bigger colonies and became an important honeybee predator in its invaded area. More knowledge on these insect's reproduction biology is requested to understand the population's dispersal and reproduction capacity and to adapt the control methods.

We compared morphology traits and fertility traits of queens of *V. crabro* and *V. velutina* in France. All captured queens were mated (n=204). *V. velutina* had twice the number and half the length of *V. crabro* sperm, in a smaller spermatheca. Ovarian maturation occurred one month earlier in *V. velutina. V. velutina* queens precocity and high fecundity may have favored this species over *V. crabro*, and facilitated its rapid invasiveness in Europe.

In the lab, the sexual maturation of *V. velutina* males is 10 days delayed after emergence. Testis show a synchronous spermatogenesis and a sperm production in large excess compared to the spermathecal content in females. Males emerging in spring -probably diploid- were compared to regular autumn males, and spring ones are far less fertile. The male fertility pattern is compatible with individual male's competition for females. A key point for the reproductive success is the synchronicity between male and female sexual receptivity.

Reproductive and behavioural correlates of dominance hierarchies in queens in the facultative polygynous ant *Myrmica scabrinodis*

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In the social system of ants, there are some genera which preserved monogyny as the ancestral social system, while some of them developed a novel social system during their evolution: polygyny. Although polygyny has many advantages, as it ensures faster growth, larger size and increases the lifespan of colonies, it also decreases relatedness within the colony, and it could lead to conflicts among queens. The status of a queen within the hierarchy could be reflected both by its reproductive conditions and by the workers' behaviour towards it. Colonies of the ant species Myrmica scabrinodis can be monogynous or polygynous, as well. The species is also parasitized quite frequently by the ectoparasitic fungus Rickia wasmannii. During our study we investigated the egg laying rate, ovary status and sperm parameters of queens from mono- and polygynous situations, and also the workers' behaviour towards queens in laboratory conditions. Our results suggest that fungal infection had a slight effect on the reproductive features, and it influenced the behaviour of workers towards queens. The type of the social system also influenced some of the measured variables, both regarding reproductive status and behaviour. This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS - UEFISCDI, project number PN-II-RU-TE-2014-4-1930.

Live long and prosper - is chromosome maintenance implicated in the extraordinary longevity of termite kings and queens?

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Longevity and fecundity have competing demands and are usually negatively correlated. A striking exception to this trend can be found in reproductives of social insects, in which a high lifelong fecundity is coupled with extraordinary lifespan. While the adaptive value of postponed senescence and prolonged reproduction stems from the eusocial organization itself, the proximate mechanisms remain elusive. Attrition and shortening of telomeric ends of linear chromosomes due to end-replication problem and stress is a well-established and ubiquitous factor limiting the longevity of cells and tissues in eukaryotes. The maintenance of chromosomes by telomerase is a widespread mechanism compensating for telomere attrition. It is linked with extension of cell proliferative capacities and extensively documented namely in vertebrates to be coupled with longevity at both tissue and organismal levels. However, chromosome maintenance through telomerase has only rarely been investigated in insects with respect to its role in preventing the ageing of somatic tissues in long-lived insect forms, such as reproductives of social insects.

We show that the telomerase activity is dramatically upregulated in both germinal and somatic tissues of termite kings and queens when compared to their sterile nestmates and document the impact of this increased activity on telomere lengths in individual tissues. Remarkable increase of telomerase activity has been observed in the somatic organs of the abdominal cavity and heads in both reproductive forms, the primary as well as the secondary (neotenic) reproductives, suggesting that the recruitment of the telomerase-driven chromosome maintenance may contribute to the extended longevity of these castes.

Symbiont mediated aging and fecundity in a fungus farming ant system

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Mycocepurus smithii is the only attine ant known to reproduce through thelytokous parthenogenesis, resulting in genetic identical queens and workers and the absence of males. The fungal symbiont, which is cultivated by the ants as their only food source, also reproduces asexually (without spores), and depends on daughter queen emergence for propagation. Preliminary data shows that, although being genetic identical, queens and workers differ in their life spans. Cross fostering experiments have demonstrated that colony performance and productivity are dependent on matching ant and fungal symbiont lineages. When cross fostered into a mismatching ant /fungus lineage combination, colony performance, productivity and fecundity declined. The double asexuality makes *M. smithii* and its fungal symbionts an ideal model system to study ultimate and proximate mechanisms of symbiont mediated aging and fecundity.

The effect of extrinsic mortality on the evolution of aging phenotypes in social insects

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Extraordinarily long lifespans of queens in eusocial insects and the striking differences in life expectancy between workers and queens challenge our understanding of the evolution of aging and at the same time offer the opportunity to study the causes underlying the adaptive variation of lifespan within a species. Variation in extrinsic mortality is widely regarded to be the main factor for the evolution of species-specific senescence patterns and variation in lifespans across the tree of life. While adaptations such as shells or wings can decrease the level of extrinsic mortality, social insects live in colonies that act as social fortresses to provide protection from extrinsic mortality. As a result, extrinsic mortality in social insects is task-, caste- and age-dependent and not random as assumed by most aging theories. To understand the evolutionary consequences of state-dependent extrinsic mortality in eusocial systems we used evolutionary individual based simulation models to investigate if extrinsic mortality can act as the driver for the evolutionary divergence of caste-specific aging phenotypes. In our models, colonies grow and provide increasing shelter from extrinsic mortality for individuals inside the colony, while foragers face higher levels of extrinsic mortality, comparable to solitary insects. Individuals in the model contain caste and age specific genes that affect intrinsic mortality rates. It turns out that a caste-specific mutation-selection balance evolves, which causes higher intrinsic mortality rates in workers than in queens, but the evolved differences between castes cannot explain the extreme divergence in lifespan between castes that is often observed in nature.

Flexibility of testis degeneration in *Cardiocondyla obscurior* males

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Males of social Hymenoptera suffer of testis degeneration shortly before or after eclosion. Mating is often a short episode on the wing during a nuptial flight. Chances to mate multiply are low, and the sperm suffices only for a few copulations.

In the ant genus *Cardiocondyla* mating takes place in the nest and sexuals are produced regularly, allowing multiple matings. This has led to the evolution of an environmentally induced male diphenism. Wingless males ("ergatoid") stay life-long in the nest and fight with rivals to monopolize all queens in the colony. As an adaptation, their testis do not degenerate and spermatogenesis continuous throughout their lives. In *C. obscurior*, winged males are produced as disperser morph under stressful conditions, and, as typical for ant males, their testes degenerate with reaching sexual maturity. However, winged males mate also within the nest before they disperse after several days, and it has been shown that the timing of dispersal depends on the availability of virgin queens and competitors inside of the colony.

Here, we investigate whether - in accordance with the behavioral plasticity - the timing of testis degeneration in winged *C. obscurior* males is flexible, dependent on their mating opportunities. We compared the expressions of germline stem cell specific genes (e.g. vasa and nanos) of winged and ergatoid males of different chronological ages with and without access to virgin queens and queen pupae as well as occurrence of competitors.

Effects of reproductive activation on longevity in *Bombus terrestris* workers

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In eusocial insects, queens and workers differ remarkably in longevity, with queens in some species outliving workers by a factor of sixty-fold or more. According to the evolutionary theory of ageing, reproduction has a causal role in generating such differences in ageing schedules. Indeed, theory predicts that disruptive selection should act to reduce the life span of helper phenotypes and increase the life span of reproductive phenotypes. However, queen and worker phenotypes differ in many ways (developmental, morphological and behavioural) that conceivably affect ageing. Therefore, the predicted causal role of reproduction in generating longevity differences has not been fully isolated and tested in eusocial insects. While queen-worker comparisons cannot, therefore, be fully conclusive, comparisons of reproductive and non-reproductive workers do not involve such confounding variables and so could prove to be more informative. Exploiting the fact that reproduction in workers of the bumblebee Bombus terrestris is readily experimentally inducible, we used workers in this species to test experimentally the hypothesis that a difference in reproductive status alone can generate differences in the ageing schedule between reproductive and non-reproductive phenotypes. Workers were randomly assigned to one of two social treatments, one of which was designed to induce reproductive activity and the other to inhibit it. The behavioural activity and longevity of workers were then monitored throughout the experiment. In this talk I aim to present the key results from this survival experiment and to discuss them in light of hypothesized relationships between the evolution of sociality and the evolution of ageing.

Gene expression profiles in the termite *Cryptotermes secundus* reveal caste specific responses to senescence.

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In many animal species a trade-off between reproduction and self-maintenance exists because limited resources can only be invested in one of the two traits, which leads to either long lifespans and few offspring or short lifes with a higher fecundity. Eusocial insects challenge this widespread longevity/-fecundity trade-off by having long-lived reproductives with many offspring. For a better understanding of the apparent lack of the longevity/-fecundity trade-off in the wood dwelling lower termite *Cryptotermes secundus* (Kalotermitidae), whole body transcriptomes of old and young individuals from three different castes (queen, king and worker) were compared. Based on gene expression patterns, individuals of the same caste clustered together. Overall, gene expression patterns in kings and workers were more similar to each other than to queens. Kings did not show a unique profile of differentially expressed genes rather profiles overlap with queens or workers depending on the age. Queens and workers on the other hand did show some genes that were always differentially expressed in comparison to the other castes. Many differentially expressed genes in old versus young individuals were involved in oxidation-reduction processes and the expression patterns of these genes were often caste specific. The overall trend for oxidation reduction related differentially expressed genes in old compared to young individuals was that they were down-regulated for queens and up-regulated for workers. These results suggest that oxidative stress plays an important role in *C. secundus* senescence processes and that the response varies between castes.

The physiological basis of the fecundity-longevity trade-off in *Drosophila* as compared to eusocial insects

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In most organisms reproduction trades off with survival. At the physiological level, this trade-off is mediated by hormones with opposite effects on reproduction and somatic maintenance. In many insects, this regulation is achieved by an endocrine network that integrates insulin-like/IGF-1 signaling (IIS), juvenile hormone (JH) and the yolk precursor vitellogenin (Vg) (or, more generally, yolk proteins [YPs]). Downregulation of this network promotes somatic maintenance and survival at the expense of reproduction. Remarkably, however, queens of highly eusocial insects exhibit both enormous reproductive output and longevity, thus escaping the trade-off. Although the causes underlying this “uncoupling” of the trade-off in social insects remains poorly understood, recent evidence suggests that highly eusocial insects might be able to defy the trade-off by having evolved a different “wiring” of the IIS-JH-VG/YP circuit. To test this hypothesis, we aim to analyse the transcriptome and endocrine physiology of sterile (germline-less) long-lived *Drosophila* – previously shown to exhibit reduced IIS – and to compare the resulting data from this solitary insect with equivalent data from bees, ants and termites collected by our collaborators.

Determinants of queen fitness: Fecundity versus somatic maintenance

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Organisms are assumed to allocate available resources to the three fundamental features of life, namely basic metabolism, reproduction and somatic maintenance, for maximizing their fitness. Reproduction and somatic maintenance are traditionally considered to be in competition with each other. However, more recently it becomes clear that aging and investment into reproduction and somatic maintenance follows species-specific trajectories and that trade-offs cannot be generalized. In contrast, this view suggests that mortality and fertility may not be strictly and causally correlated after all. In social insects the view that reproduction is costly per se has also been challenged on the grounds that queens in general live much longer than non-reproductive workers, so high fecundity per se does not necessarily limit lifespan. Here we aim to study the relation of aging with the costs of metabolism, fertility and somatic maintenance using the emerging social insect model Cardiocondylaobscurior. We will study whether and to what extent caloric restriction and investment into reproduction translate into pathways implicated with aging using a combination of RNAseq and metabolomics.

Mating, immunity and longevity in ant males

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Across multicellular organisms the costs of reproduction result in a life history trade-off between fecundity and longevity. Notable exceptions to this rule are queens of perennial social Hymenoptera that are both highly fertile and long-lived, but at the same time seem to successfully avoid this classical trade-off. However, it remains largely unknown how reproduction affects the lifespan in social insect males, as mating typically occurs in unobservable mating flights and hence, data on male longevity and / or reproductive success are difficult to obtain. In the ant genus *Cardiocondyla*, wingless males mate inside their maternal nest with young virgin queens, stay lifelong in the colony, and reach extraordinary long lifespans compared to other social insect males that often die on the wing. This allows investigating the classic trade-off between fecundity, longevity and immunity, the latter being further predicted to be a costly life history trait. In this study, we compare longevity, reproductive success, and immunocompetence of *C. obscurior* males kept virgin or having mated with a low and high number of virgin queens, respectively.

Convergent evolution of longevity in eusocial cockroaches and Hymenoptera

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A hallmark of aging is the trade-off between longevity and fecundity. However, reversals of this trade-off and the concomitant escape from aging have convergently evolved in taxa as distantly related as the social hymenoptera (bees, ants, wasps) and the social cockroaches (termites). Indeed, the lifespan of queens and kings (in termites) that monopolize reproduction within insect societies can exceed that of sterile individuals by up to two orders of magnitude. A recent surge in genomic resources has boosted studies into the molecular mechanisms underlying the reproductive division of labour among the social hymenoptera; yet, how the longevity/fecundity trade-off changes with sociality has received less attention. Moreover, the termites have been largely neglected, despite their independent evolutionary origin of similar lifespan divergence between reproductive and non-reproductive individuals. We aim to identify the molecular toolkit underlying the reversal of the fecundity/longevity trade-off in social insects, focusing on both hymenoptera and cockroaches. Our comparative analyses include the first genome of the non-social, close relative of the termites, the German cockroach (*Blattella germanica*). This approach will gain a deeper understanding into the molecular, evolutionary and genetic basis of how social insects escape the longevity/fecundity trade-off in distantly related taxa.

Social behaviour and the reversal of a life history trade-off in a facultative social orchid bee

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Organisms are typically faced with a trade-off between their fecundity and longevity, as they must choose between allocating limited resources to reproduction or to their own maintenance and survival. Eusocial insects constitute a notable exception to this rule, with social queens not only exhibiting much longer lifespans than workers, but also producing the majority of their colony's offspring. This positive association between lifespan and fecundity in eusocial insects suggests that a reversal of the fecundity/longevity trade-off is linked with the evolution of eusociality; however the mechanisms underlying this reversal are still poorly understood. The orchid bee *Euglossa viridissima* is a socially polymorphic species, meaning that individuals within this species can exhibit either solitary or social behaviour, which makes it a perfect model system for investigating the proximate causes underlying the reversal of the fecundity/longevity trade-off in social individuals, in comparison with their solitary counterparts. Following a semi-field design whereby females were artificially induced to nest in wooden boxes, 24 nests were observed over a five month period in order to characterise the behaviour of solitary versus social individuals and to sample females from different social castes (solitary, worker, queen) for subsequent comparisons of gene expression and hormone titres. Here, preliminary data on the nest establishment patterns and the social behaviour of the species are presented. These results provide us with a better understanding of the degree of sociality exhibited by *E. viridissima*, and help us make predictions as to the effects of sociality on the fecundity/longevity trade-off in this species.

Does arriving first give you right to rule?

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Many social groups are organised in hierarchies, the dominant position providing access to the major share of reproduction and thus fitness. In primitively social species, all individuals can take on the dominant role, but only some do - raising the question of how this hierarchy is established. The primitively social Polistes paper wasps are a model system for this research, as their open combs make it practical to observe foundress behaviour and brood development. P. dominula foundresses start nests singly or in groups, most of which gain joiners. After an unstable period, a hierarchy is established. The likelihood of securing rank 1 will depend on the rules used to establish the hierarchy. Previous work suggests order of arrival may be important in other paper wasps, but this remains uncertain in P. dominula. Using a population in Spain we marked the first arriving wasp at each nest and later, upon establishment of the hierarchy, determined its rank. We found that first-arrivers are more likely to be dominant than expected by chance. We also explore the importance of other covariates. It is possible that a hierarchical system based on order of arrival arises because it is less vulnerable to disruption following each joining event: the first female remains rank 1. It is also conceivable that early arrival may be a measure of quality. Our finding, that the first to arrive gains the right to rule provides exciting possibilities for future research, as we can predict the future dominant early in the season.

Relationships between prevalence of the symbiont *Wolbachia* and sex, caste, colony size and colony productivity in *Temnothorax crassispinus*

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The reproductive parasite *Wolbachia* is widespread across social insects. In spite of this, we know very little about the basic biology of *Wolbachia* infections in insect societies. Here, we aim to elucidate the factors underlying patterns of infection within and across colonies of the temperate ant *Temnothorax crassispinus*, and determine whether *Wolbachia* infections are associated with host fitness. We collected 88 *T. crassispinus* nests and conducted a complete census of each colony. In addition, we used diagnostic PCR assays to screen workers, gynes and males from each colony for the presence of *Wolbachia* to gain an estimate of the prevalence of infection within and across sexes, castes and colonies. Our findings indicate that *Wolbachia* is extremely common within this population, with 97.7% of colonies harbouring at least some infected workers. In addition, we found a strong positive relationship between colony size and the prevalence of infection within the worker caste. Our analyses also suggest that, whilst infection has no measurable impact on colony sex-ratios, colonies with a greater proportion of infected workers were more productive. Finally, we uncovered considerable differences in the infection rates of different castes; gynes were more likely to be infected than workers (89.4% vs. 70.2%) and workers in turn were more likely to be infected than males (54.9%). These intriguing relationships between *Wolbachia* prevalence and sex, caste, colony size and colony productivity merit further investigation.

Social structure and colony size, but not body size influences fecundity in the ant *Temnothorax rugatulus*

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Life history traits such as fecundity and lifespan are under strong selection as they directly affect fitness. The common trade-off between fecundity and longevity are reshaped in social insects, but it remains unclear which factor affect fecundity of ant queens. The social structure of their colonies, their body size, colony size or the worker care, which they receive? We tested the impact of these factors and their interactions on individual queen reproduction in the ant Temnothorax rugatulus, in which two queen morphs occur: large macrogynes mostly residing in monogynous colonies and the smaller microgynes from polygynous colonies. We experimentally manipulated worker and queen number and observed queen egg-laying rates and worker care. Individual queens in polygynous colonies reproduced less than those in monogynous ones, when controlled for worker number. Though reproduction in polygynous colonies was skewed, all queens laid eggs. Queen fecundity was limited by worker number as queens had higher reproductive rates in larger colonies, probably due to more worker care. Interestingly, egg-laying rates did not differ between the two queen morphs. However, worker cared for them differently. Albeit they were less often groomed, smaller microgynes were more often fed, possibly explaining why they laid eggs at the same rate as macrogynes. Queen body size did not affect fecundity or reproductive skew later in mature colonies. We are currently comparing brain and fat body transcriptomes of founding and established queens to identify genes involved in fecundity and longevity and we present the most interesting candidate genes.

Why do leaders ditch societal investment during emergency? Brood transport in an Indian queenless ant

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Animal groups move to secure resources or to avoid hazards. During relocation process they may pay the cost of losing their young ones. While most ants use chemical trails to move to the new nest, the model system for this study, Diacamma indicum, employs tandem running. This is relatively primitive mode of transport consists of a stereotypical behaviour, in which an informed individual (tandem leader) leads a follower ant to the new nest. Thus tandem leaders play an important role during the relocation process as they are responsible for the both adult and brood transport. We examined how the rescue of brood items occurs at the level of the colony, and at the level of individual ants. Among the brood items i.e. eggs, larvae and pupae, pupae represent the most valuable but the least vulnerable item while larvae represents the less valuable but relatively more vulnerable item. Given that we asked if ants show preferential rescue of their investment as compared to most vulnerable items. Even though there was no preferential rescue at the colony level, individual choice test of workers showed a clear preference. Followers showed a preference for vulnerable item while tandem leaders preferentially rescued highest investment. Further we found that tandem leaders' relax such preferences in the presence of nest mates. How behavioural difference among tandem leaders and followers during emergency and condition-dependent choice of tandem leaders' helps to makes this primitive mode relocation an efficient process would be presented in this study.

A New Kind of Ant Trail Achieves Efficient Routing in Error-Prone Conditions

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The mass recruitment pheromone trail is of the most magnificent collective behaviors displayed by ants. While such trails occur in relatively stable environments, ants that cooperatively transport large food loads must perform under conditions of increased uncertainty. These can result, for example, from the limited knowledge individual ants might have regarding the admissible routes from the point of view of the much larger load. Learning how ants have evolved to utilize scent marks while navigating in such error-prone conditions can be beneficial from both a biological and an engineering perspective. Using a novel methodology for the detection of pheromone deposition by ants, we discovered that, in the context of cooperative transport, ants employ scent marks in a manner that significantly deviates from the classical notion of an ant trail. Specifically, this new kind of ant trail is characterized by short broken segments that are dynamically redrawn in front of the moving load. Furthermore, the ant-load complex occasionally ignores scent marks and loses the trail which is then reassembled from another location. We algorithmically analyzed these trail-laying and trail following principles using an abstract routing model on graphs to prove that, for a relevant family of graphs, the resulting expected travel time is optimal, even in the presence of possible errors in scent laying directionality. We provide experimental evidence for similar efficiency in the ants' collective motion.

Neuroactive compounds associated with behavioural transitions during colony foundation in the ant *Lasius niger*

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Across species, ant queens present a wide variety of modes of colony foundation. In the black garden ant Lasius niger, freshly mated queens can either found their colony alone (haplometrosis) or in association with one or more co-foundresses (pleometrosis). An intriguing characteristic of multiple queen foundation is that queen partnership breaks down after the emergence of the first workers and all but one foundress are eliminated during violent fights. This abrupt behavioural transition suggests the existence of an underlying physiological mechanism possibly mediated by neuroactive compounds. Octopamine is a biogenic amine that has often been associated with aggression in insects. Inotocin, on the other hand, is the insect ortholog of oxytocin, a mammalian neuropeptide involved notably in social bonding. Inotocin might therefore influence similar behaviours in insects; however to date its role remains largely unknown. Combining gene expression quantification and behavioural observations, we studied the evolution of octopamine and inotocin levels over time in haplometrotic and pleometrotic queen brains. Our results show significant gene expression variations associated with major behavioural transitions during the first months of colony foundation.

Molecular mechanisms of high mortality in social isolation

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Social deprivation can have negative effects on the lives of social animals, including humans, yet little is known about the mechanisms by which social withdrawal affects animal health. In the carpenter ant Camponotus fellah, socially isolated workers have a greatly reduced lifespan relative to ants kept in groups of 10 individuals. By using a tracking system, we found that social isolation resulted in important behavioral changes and greatly increased locomotor activity. The higher activity of single ants and their increased propensity to leave the nest to move along the walls suggested that the increased mortality of isolated ants might stem from an imbalance of energy income and expenditure. This view was supported by the finding that while isolated ants ingested the same amount of food as grouped ants, they retained food in the crop, hence preventing its use as an energy source. Furthermore, we performed the transcriptome analysis to compare the gene expression between grouped and isolated ants that were separated from the mother colony. We found that the gene expression profile of isolated ants is similar with that of other organisms which show short lifespan in aged or stressed condition. We would like to discuss about the molecular mechanisms how the social environment affects the fitness of ants from the transcriptome analysis.

Phenotypically normal worker honey bees (*Apis mellifera capensis*) with three alleles at multiple microsatellite loci

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We generated reciprocal crosses between two honey bee subspecies *Apis mellifera capensis* (hereafter Capensis) and *A. m. scutellata* (hereafter Scutellata) using single-drone artificial inseminations. Workers and virgin queens of Capensis are thelytokous (i.e. their offspring are female parthenogens), whereas Scutellata are arrhenotokous (i.e. their offspring are male). We genotyped the queens, the fathering males and the worker progeny of these crosses using microsatellite markers. In colonies with a Capensis queen, but not in colonies with a Scutellata queen, we found workers that carried the two alleles of their mother as well as an allele inherited from their father. In another experiment we found that Capensis queens inseminated with Capensis males produced eggs that carried three alleles, two from the mother and one from an inseminating male.

The progeny were phenotypic females with no evidence of gynandromorphy (male and female tissue in the same animal). Two explanations of these individuals are plausible. First, they may be triploid and express female traits because of heterozygosity at the sex locus. Second they may be female mosaics arising from two zygotes that formed from two maternal pro-nuclei each of which fused with a different sperm. The consequences of these findings for the social structure of Capensis colonies will be discussed.

Rolling behaviour in a cryptic ant: when escape is the best defence

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The evolution of efficient defence systems is one of the features behind the success of arthropods. In this context, both solitary and social species are equipped with chemical, mechanical and behavioural weapons allowing an effective protection from sources of danger. Ants offer a large sample of sophisticated defensive strategies. These insects, in addition to adopting social protection (alarm and cooperative defence), may show strategies similar to those of solitary animals. For example, some species react with individual aggression to attacks or adopt freezing behaviour. In other cases, escaping may be the most ecologically appropriate system of defence. Here we report a first detailed description of a peculiar escaping strategy adopted by *Myrmecina graminicola*, a cryptic ant whose colonies live in the forest floor. By field and laboratory investigation, we verified that in the presence of disturbance (aggression, mechanical stimuli, strong vibrations), workers of *M. graminicola* may respond with immobility or simply moving away. However, in other situations (e.g. specific inclinations of the substrate, vertical dropping), the ants behave in a very different way: they promptly fold in on themselves, lay their appendages close to the body and actively roll up to escape. Once the ants stopped rolling, they suddenly resume their normal position and run away. In this way, they can rapidly escape from a potential danger and hide under leaves and ground slits. This is a novel defensive behaviour discovered in ants and represents an interesting context-dependent strategy characterised by specific morpho-functional and behavioural adaptations.

Determinants of a novelty-seeking behavioural syndrome in the Argentine ant and evolution along an introduction pathway

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Are we driving the evolution of invasive species to our own detriment? Humans routinely transport invasive species around the globe. It has been suggested that this process leads to selection for traits that enhance invasiveness. We are testing this hypothesis by investigating a novelty-seeking behavioural syndrome in the Argentine ant, which has successively colonised most subtropical regions around the world. Novelty-seeking, a risk-taking behavioural syndrome, is seen as a major component of invasiveness in animals and is thus likely to be under strong selection in invasive species.

We studied variation in novelty-seeking behaviour in native as well as introduced Argentine ant populations from California, Australia and New Zealand in order to determine if data was consistent with selection along the introduction pathway. We are combining behavioural assays, neurochemical and transcriptomic analysis to investigate further the behavioural syndrome as well as its molecular basis. Our first results show that octopamine – an important biogenic amine – mediates novelty-seeking behavioural syndrome both at the individual and colony levels. Behavioural variation within regions was high, but we found no phenotypic evidence for increased novelty-seeking along the studied introduction pathway.

The next step of this study is to perform a global comparative analysis of the Argentine ant transcriptome in the different regions along its introduction pathway. This will help to investigate underlying mechanisms of how evolution and invasion processes may interact, as well as to understand better the molecular basis of variation in a key behavioural trait in the invasive Argentine ant.

Linking supergene to complex social phenotype: a search for the behavioral processes controlling colony queen number

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Supergenes regulate a variety of complex phenotypes, from batesian mimicry in Heliconius butterflies to divergent male reproductive morphs in the ruff. In the ant Formica selysi, alternative genotypes at a supergene are associated with the social structure of the colony. The supergene thus controls whether colonies are headed by a single or by multiple queens. However, the ontogenetic processes leading to this complex colony-level social phenotype are not yet known. In a series of field and lab experiments, we search for key behavioral mechanisms that are susceptible to regulate colony queen number. First, we examine if workers determine the number of queens in their colony by accepting or rejecting supernumerary young mated queens. Second, we test if variation in colony queen number is caused by differences in mating behavior and dispersal of young queens and males. The unveiling of the behavioral mechanisms causing alternative social organizations will provide a key link between supergene and complex social phenotype in the ant Formica selysi, and will further reveal the role of supergenes in social evolution.

Bacterial community composition in a socially polymorphic ant

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Understanding the factors that govern host associations with microbes has been a long-standing challenge in microbial ecology and evolutionary biology. Formica selysi is an interesting model system to study the impact of host social organization on bacterial communities. This ant species has colonies headed by a single-queen (monogynous) and colonies headed by multiple queens (polygynous) in the same population. We used 16S rRNA Illumina Sequencing to characterize the bacterial community composition of monogynous and polygynous colonies. We then compared bacterial communities across colonies and between social forms. Bacterial communities varied greatly across colonies. Two common groups of OTUs (Operational Taxonomic Units) belonging to the Burkholderiales and Enterobacteriales, respectively, showed antagonistic distribution across colonies. The bacterial communities of monogynous and polygynous colonies differed significantly. Overall, restricted exchanges among colonies and between social forms, coupled with antagonistic interactions among bacteria species, appear to shape the dynamics of ant-microbial associations.

Journey to the heart of termites' family life: An uncommon inner insight into nascent colonies

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In social insects, the foundation phase constitutes a bottleneck event and thus, a critical step for incipient colony development. If processes originating this survival pitfall are well studied in Hymenopteran, they remain unclear in termites. Indeed, their founding phase is difficult to observe as royal couples seal themselves in a nuptial chamber right after choosing a suitable nestsite.

In our study, we optimized a non-destructive apparatus permitting to observe the evolution of more than 150 incipient colonies weekly over six months after queen and king crossing. Two European species were here compared: the invasive Reticulitermes flavipes and the French native R. grassei. An overview of couple fitness proxies (egg laying rate, caste development and survivorship) and behavioral items of each caste (queen, king and descendant) were obtained. These informations provide a complete insight of the foundation process and family life of these termites. Overall, four main results arised from these data. A higher foundation success and a differential brood caring strategy were observed in R. flavipes' incipient colonies. Agonistic behaviors between queens and kings were higher in R. grassei's. Moreover, investment of parents in nest upkeep differs in both species shedding light on a surprising paternal investment in reproductive success.

An interesting correlation between fitness and behavioral data shaped from these results and varies between species. This suggests differential foundation strategies which may underlie their invasive and native status. To complete our understanding of subterranean termite foundation, further studies on foundations by neotenic reproductives (budding) would be of interest.

Antennal response to odorants with innate or acquired valence in honeybees (*Apis mellifera*)

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In order to survive, animals must produce adaptive behaviors when facing potentially positive or negative situations. In honeybees, olfactory cues are highly relevant for communication among nestmates, foraging and nest defense. We studied the effect of odor valence on the movements of crucial sensory organs for bees: their antennae. We developed a tracking system for monitoring harnessed bees' antennal movements at a high frequency rate. The valence of an odor can be innate or acquired through learning. We analyzed (i) the spontaneous antennal response toward odors, whose valence was measured based on the spontaneous orientation of bees, and (ii) the change in odor-evoked antennal movements as a result of differential appetitive or aversive conditioning, inducing opposite acquired valence. In the appetitive conditioning of the proboscis extension response (PER), an odor (CS) was associated with sucrose solution (US), whereas in the aversive conditioning of the sting extension response (SER), an odor CS was associated with a thermal shock US. Spontaneous antennal responses to odorants seem to be separated in fast-forward or slow-backward movements. While queen and brood pheromones, as well as royal jelly odor, induced fast and forward movements, alarm pheromones induced backward movements and a decrease in velocity. Appetitive conditioning had a strong effect on antennal movements, bees responding to the odorant associated with sugar with a marked fast and forward motion of the antennae. By contrast, aversive conditioning had little effect on antennal movements. These experiments unravel the existence of both innate and acquired, valence-based, antennal responses in bees.

Group nutrition in gregarious insects

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What to eat, when and with who? Insects, like virtually all animals, carefully regulate their intake of key nutrients to reach physiological states maximising growth and reproduction. While this may seem a relatively easy task, nutritional decisions are considerably complicated by social life. In eusocial ants and bees, foragers must integrate their own nutritional needs as well as those of all other colony members, including the non-foraging workers (that primarily require carbohydrates for energy), the larvae and the queen (that require substantially more protein for growth and reproduction) to collect foods addressing a colony-level nutritional target. Whether and indeed how these complex collective regulatory dynamics are observed in socially simpler insect groups is virtually unexplored. At the most basic level, individuals may face a trade-off between choosing foods addressing their own individual needs and following others to maintain group cohesion. Here I will present models and experiments on a gregarious insect, the fruit flies Drosophila melanogaster, showing that non-cooperating foragers can use social information to better locate food resources and efficiently balance their nutrient intake, ultimately leading to collective regulatory behaviour. Using simulations of nutritionally explicit agent-based models, I will discuss new hypotheses about the role of nutrient spatiotemporal availability in the emergence of collective behaviour in groups exhibiting various levels of social complexity and across different ecological scenarios.

Novel support for the kin conflict theory of genomic imprinting in the bumblebee *Bombus terrestris*

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In many mammals and flowering plants it has been shown that some genes can show an unusual expression pattern in which only the paternally or maternally inherited copy of a gene is expressed. The kinship theory of genomic imprinting posits that such uniparental gene expression may be a consequence of the differing kin interests of matrigenes and patrigenes. In social insects, matrigenes and patrigenes are also expected to strongly differ in their evolutionary interests, thereby allowing for an independent test of the kin conflict theory of genomic imprinting. Indeed, in the honeybee it has recently been shown that vitellogenin, the ecdysone receptor gene and ecdysone induced protein 75 are all paternally expressed in reproductive workers, which was consistent with these genes being reproduction-enhancing and with patrigenes favouring greater worker reproduction. This prediction was made on the basis that in the social Hymenoptera, the father's genes are only transmitted through the sons of the workers but not through those of the mother queen. Here we present new independent evidence in support of the kin conflict theory of genomic imprinting in the bumblebee Bombus terrestris. Based on reciprocal crosses between two Bombus terrestris subspecies and parental genome and transcriptome sequencing, we carried out a genome-wide screen for imprinted genes in reproductive and sterile workers. Surprisingly, we found that ca. 100 transcripts showed a clear paternal expression bias. In addition, we found that the top imprinted gene, which was 98% paternally expressed, showed homology to farnesol dehydrogenase, which is known to be involved in juvenile hormone biosynthesis, and which in bumblebee workers is linked to reproductive dominance. These results, therefore, are in tentative agreement with the kin conflict theory of genomic imprinting. Overall, however, there was no overlap in terms of which genes were imprinted or not in bumblebees and in the honeybee. This suggests that the imprinting status of genes evolves very quickly and may be subject to an evolutionary arms race.

Lateralization of behaviour in social insects - emergent adaptive benefits

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There is now an increasing amount of evidence for sensory and motor asymmetries in the behaviour of invertebrates. Population-level behavioural lateralization may be more likely to evolve in social than solitary species [1]. Alignment of the direction of behavioural asymmetries is favoured as an evolutionarily stable strategy when asymmetrical individuals must coordinate their behaviours [2]. However, evidence for lateral biases in ants and other social insects is relatively limited, though their eusociality makes them inviting subjects in which to investigate this hypothesis.

I have previously studied the nest site exploration behaviour of *Temnothorax albipennis* ants and found a leftward bias, with about 65% of individuals choosing a left turn as they entered the nest cavity [3]. A population-level bias may be adaptive owing to enhanced group cohesion, reducing predation risk (‘safety in numbers') and speeding up the nest emigration process.

I will present the results of recent fieldwork in Sydney, Australia, examining the behaviour of the meat ant *Iridomyrmex purpureus* as it encounters an unexpected barrier blocking a foraging trail. A rightward turning bias is found (60% preferring rightward turns), and in this context adaptive value may similarly be derived from enhanced group cohesion and social resilience.

Lateralization of behaviour, rather than being incidental, is argued to have potential adaptive benefits for social insects generally, and is something worth routinely considering in many behavioural experiments going forward.

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Specificity and stability in an obligate, vertically transmitted symbiosis: an experimental analysis

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A key feature of symbioses is stability and persistence across ecological and evolutionary scales. Although the socalled ‘higherattini’ (the leafcutter ants (Atta and Acromyrmex spp.) and nonleafcutters (Trachymyrmex spp.) were widely considered to be a classic case of 1:1 coevolution because each of these two groups of ants grow distinct clades of derived ant fungi, large scale geographical surveys have shown that there are naturally occurring symbiont switches between these two ant and fungal clades. The mechanisms that promote or maintain these associations are currently unknown. Reported here are the results of experimental symbiont switching (crossfostering) from six North American fungus gardening ant species. The six species show three generalized phenotypic responses when growing novel fungi. Some fail immediately, some sustain growth for several weeks before undergoing irrevocable declines, while others remain stable for 4 years or more. Because the unstable combinations become invaded by weedy, pathogenic species, it is thought that ant and fungal microbiomes may have a role in stabilizing ant and fungal combinations over ecological and evolutionary time scales.

Early experience does not shape social performance in spiderlings

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The ability to shift toward cannibalistic behavior represents a central mechanism responsible for the maintenance of populations facing low prey availability. In many organisms this behavioral transition is favored by morphological adaptations facilitating the capture and consumption of conspecifics. Interestingly, some species are reluctant to cannibalism at certain ontogenic stages despite their ability to prey on conspecifics. Spiders belong to these particular species, since all juveniles show a transient gregarious phase that extends after their emergence from the maternal cocoon and ends with the initiation of agonistic interactions and cannibalism. Most studies that investigated social interactions in spiderlings focused on kin recognition. However, the potential mechanisms shaping recognition and discrimination, and particularly the role of early social experience, received little attention. Therefore, this study examined to what extent early social isolation can influence the expression of tolerant and predatory behaviours. We also investigated the influence of familiarity and kinship, and of their interaction with social experience, on the expression of cannibalism. Our results showed that social isolation of spiderlings of *Agelena labyrinthica* since the egg stage did not influence social performance after hatching. Our study advocates for the existence of a species-specific chemical signature inhibiting cannibalistic tendencies in spiderlings.

Insights into bumblebee fossils

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One of the main challenges in paleontology is to integrate fossils in the taxonomy and phylogeny of extant clades. Such fossils taxonomy remains too often based on the description of a few qualitative and subjective characters whereas the use of robust statistical tools is quite rare. Herein we underline the importance of such tools by presenting a taxonomic revision of bumblebee fossils based on reliable statistical analyses. Wing-shape analysis by geometric morphometrics procedures has proven to be a powerful tool for morphological discrimination of bee taxa at different taxonomical levels. Moreover bee forewings are easy to analyze because of their flatness and represent a neutral character with enough variation to discriminate taxa. Previous studies have shown the utility of wing shape analysis to assess taxonomic affinities between extinct and extant clades of bees. Bees are relatively rare in fossil deposits; nonetheless using this method, anthophorids, halictids, bumblebees and other clades have been revised or described from different deposits like the Florissant shale of Colorado (US) or the Eocene Baltic amber. For the present analyses, three bumblebee fossils have been considered: Bombus randeckensis Wappler & Engel, 2012 from the Miocene Randeck Maar (DE); Bombus cerdanyensis Dehon, De Meulemeester & Engel, 2014 from the Eocene of the Green River Formation of Colorado (US); and a new specimen from the Miocene of Bilina Mine (CZ).

Crash landings: nest entrance behavior of *Partamona helleri* stingless bees

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The stingless bees (Meliponinae) construct a diverse range of species-specific nest entrances. One genus, *Partamona* has a conical ‘toadmouth' entrance, comprising wide outer and narrow inner entrances. This structure seems to have given rise to a curious behaviour in bees returning to the nest. *Partamona* bees have been observed to accelerate on their approach to the nest and ‘crash', rather than land, into the entrance. We studied *P. helleri* bees to quantify this behaviour. To put this behaviour into context, we studied two other meliponines with contrasting entrance structures: *Scaptotrigona depilis* which builds a medium-large tubular entrance, the ancestral state of the toadmouth, and *Melipona scutellaris* which has a narrow entrance. All species initially decelerated at 0.5ms^{-2} as they approached the entrance. *S. depilis* and *M. scutellaris* maintained this rate until they reached the entrance at a speed of 0.5ms^{-1}. However, at 0.2m from the entrance *P. helleri* began to accelerate at 1.0ms^{-2}, finally hitting the entrance at 1.4ms^{-1}. *P. helleri* did not land with its feet but rather crashed head first into the outer entrance and tumbled down into the inner entrance. Higher speed did not result in reduced accuracy, *P. helleri* made no fewer mistakes entering its nest than either of the slower species. Last, we used a bioassay to test whether the acceleration of *P. helleri* is a mechanism to avoid ambush predation at the entrance. The acceleration and crash landing behaviour of *P. helleri* seems to be an exaptation of its extended phenotype, the nest entrance.

Comparative proteomics reveals the evolution of seminal fluid proteins at the transition between single and multiple mating in fungus-growing ant queens

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The queens of social Hymenoptera store male ejaculates in specialized organs (spermathecae) after a single mating episode early in adult life. While single queen-mating is the ancestral state, a number of haplodiploid social insect lineages have made evolutionarily derived transitions to multiple queen-mating. This implies that ejaculate competition for ultimate storage by queens has convergently evolved multiple times and that the need for queens to control strife among sperm after storage is expected to show similar signatures of evolutionary convergence. Sperm competition has been documented at the phenotypic level in honeybees and leaf-cutting ants and seminal fluid proteins have been implicated as agents of mutual sperm incapacitation. However, the underlying molecular mechanisms and the identity of the proteins involved have not been resolved. We used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify complete seminal fluid proteomes across the crown group in the fungus-growing ant phylogeny, comparing polyandrous Atta and Acromyrmex species with a monandrous Trachymyrmex sister lineage representing the ancestral state without sperm competition. This showed that only a few percent of all identified proteins appear to have a role in sperm competition. Through screens of signatures of positive selection, we are now investigating the evolution of these proteins across additional fungus-growing ant species for which draft genomes are available, including a socially parasitic species, which has evolved from a polyandrous host but secondarily reverted to single queen-mating. Our results allowed us to formulate specific hypotheses about the molecular mechanisms underlying the evolution of sperm competition in leaf-cutting ants.

Energy Management of Commercial Bumblebees during Diapause

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Agricultural production is essentially linked to pollinators. Commercially reared bumblebees are increasingly used to pollinate agricultural plants in greenhouses, orchards, and fields. In Europe, Bombus terrestris (B. te.) is the most frequent species reared in laboratory, especially the south European subspecies B. te. dalmatinus. Commercial reared B. te. dalmatinus is distributed even in Central Europe, where it does not occur naturally. Escaping from greenhouses represents environmental risks to local species such as B. te. terrestris in Central Europe

In our study, we aimed to assess the ability of B. te. dalmatinus to accumulate enough reserves to survive the winter in Central Europe. Thus, we compared amounts of the accumulated nutrients (lipids and glycogen) and their quality in the queen's fat body of two subspecies - B. te. terrestris and B. te. dalmatinus. The lipids were extracted from fat bodies of five life stages (after eclosion, before dormancy, after hibernation, after aestivation, and egg laying). The extracted lipids including phospholipids and neutral lipids (triacylglycerols) were weighted and then analyzed by LC/MS. The amount of glycogen in the fat body was determined by a coupled enzyme assay, which produces a colorimetric product, proportional to the glycogen content. The results show only small differences between both subspecies. The amount of lipids and glycogen is changing at stages in both subspecies, reaching maximum before dormancy. Commercially reared alien B. te. dalmatinus is a potential danger for local B. te. terrestris population.

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Synergistic breakdown of plant substrate compounds by leaf-cutting ants and their fungal symbionts

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Leaf-cutting ants are regarded as major agricultural pests in Neotropical regions. These ants use freshly-cut leaves to provision a basidiomycete fungus-garden symbiont, which in turn feeds the farming ants and their brood. The emergence of ant-feeding fungal gongylidia and the use of ant fecal fluid for vectoring fungal enzymes through gardens were key innovations that initiated co-evolution in the higher attine ants. We obtained proteome profiles of the fecal fluid from large workers in four colonies of *Acromyrmex echinatior* using nanoLC-MS/MS, which extended our data set to almost 100 proteins. This confirmed that most fecal fluid proteins originate from the fungal symbiont and pass unharmed through the guts of the ants to end up in the fecal fluid for deposition in actively growing parts of the garden. However, it also showed that some of these proteins (mostly proteases) originate from the ants, and belong in part to an expanded gene family of M14 metalloproteases. RNA-seq and qPCR analysis further showed that the expression of many of the fungal-derived fecal fluid proteins is upregulated in the gongylidia, consistent with adaptive functions after deposition in the garden. Most of the identified proteins are degradation enzymes targeting plant cell wall components, but also many oxidoreductases were found. These enzymes may degrade defensive plant compounds and/or produce hydrogen peroxide for the breakdown of recalcitrant plant cell wall polysaccharides using Fenton chemistry. Our study elucidates the intricate co-evolution between leaf-cutting ants and their fungal symbiont towards utilizing fresh leaf material as a novel food source.

Fitness and Aging in *Cardiocondyla obscurior* Ant Queens

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Easy maintenance, controlled mating and short generation time make Cardiocondyla obscurior an interesting model for social insect aging research. Using this ant we have begun to study the proximate genomic relationship between mating and aging. While mating in general has a positive effect and results in highly fecund queens with long life but reduced metabolic and protein turnover rates, queen fitness strongly depends on the quality and compatibility of the mate. I will review a decade of research on factors affecting C. obscurior queen fitness and contrast these findings with studies on honeybees and solitary aging models. I will conclude with a brief outlook of what is to be expected from this model in coming years.

First characterization of the circadian clock of *C. floridanus*

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As social insects, ants belonging to the species *Camponotus floridanus* are confronted with a number of challenges that need the right timing. These challenges range from collection of nectar, to brood care, to mating. To time such behavior, they use an endogenous clock. We investigated the properties of this endogenous clock by recording the locomotor activity of individual C. floridanus ants under 12:12h light-dark cycles and constant darkness (DD). We found that most ants were nocturnal and that their activity rhythms free-ran with a period of circa 23 hours under DD. Their period was highly temperature compensated. Immunohistochemical stainings with an antibody against the clock protein PERIOD (PER) revealed PER positive cells in the lateral and dorsal brain. Few PER positive cells in the lateral brain contained the neuropeptide Pigment Dispersing Factor (PDF). The amount of PER cycled significantly in most neurons, but not in the PDF-positive cells. PDF-staining furthermore revealed the neuronal network of the endogenous clock in the brain of different morphological castes of *C. floridanus*.

Comparing behavior and properties of real and simulated honeybees controlled by the BEECLUST algorithm

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Honeybees are known for their wide range of collective behavior, which allows them to complete complex tasks, single honeybees would never be capable of. What astonishes the most is that these tasks can be executed, in spite of the physical limitation of individual honeybees. We can hypothesize about different ways of optimization the honeybee colony relies on, however it is complicated to prove these hypotheses. To give an indication about validity, we compare here data generated on basis of our hypothesis with empirically gathered data. We established experiments for testing two different aspects of collective behavior relating the BEECLUST algorithm. This collective-decision-making algorithm, founded on the basis of honeybee behavior, shows a possible solution on how to successfully perform a complex task in a dynamic environment with limited resources. Millions of years of evolution resulted in well adapted honeybee colonies, for example with (assumingly) near optimal group composition and social interaction. We performed neural network driven optimization experiments with simulations and robots, representing honeybees and their group composition as well as experiments with real honeybees. In addition to that, we analyzed a different social aspect of the BEECLUST algorithm, the effects of social gradients. Without modifying the source code, introducing a social gradient affects robots qualitatively the same way as bees. Thus we can show that our results obtained through simulations qualitatively resemble empirically obtained data from real honeybees.

Evolving Eusociality: Using *Drosophila* to understand how queen pheromone inhibits reproduction in worker honeybees

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This work aims to understand the evolution of eusociality (the social structure where a dominant caste reproduces, and the repressed caste rears the offspring) in honeybees, particularly how queen mandibular pheromone (QMP) induces reproductive constraint in the worker. We are using the easily manipulable Drosophila melanogaster to investigate this process.

Newly emerged virgin female Drosophila were exposed to synthetic QMP for 48 hours, their ovaries removed and mature oocytes counted as a measure of fecundity. QMP exposure caused a significant reduction in the number of mature oocytes in a dose responsive way. Drosophila were exposed for various periods QMP, to generate a time-course across ovary development. This showed that the number of mature oocytes was reduced significantly from 24 hours onwards. We have demonstrated that this response is plastic and reversible by removing Drosophila from QMP and allowing ovarian development to proceed, leading to a significant recovery of phenotype.

RNA-seq is currently being carried out on ovaries from QMP exposed Drosophila across these time points, as well as those with recovered phenotypes. This allows investigation into which genes are showing altered expression during this process.

The non-social and highly-diverged Drosophila responding to QMP from the eusocial honeybee gives insights into the evolution of this social structure. It raises the possibility of a conserved mechanism of responding reproductively to environmental cues, which may have been co-opted into a novel role in this eusocial species. Drosophila provide an excellent genetic tool to further understand this process.

The Molecular Basis of Cuticular Hydrocarbon Detection in the Ant *Harpegnathos saltator*

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Ants and other eusocial insects have co-opted a subset of the cuticular hydrocarbons (CHCs), generally used as a desiccation barrier, to function as pheromones mediating several key social behaviors such as caste and nestmate recognition. Furthermore, CHCs mediate the reproductive division of labor, with queen-specific CHCs acting to repress ovarian development in workers. Using the ponerine ant Harpegnathos saltator, we investigated a greatly expanded subfamily of odorant receptors (HsOrs) and characterized them as CHC receptors. Through functional expression in transgenic Drosophila, we systematically tested 22 HsOrs, observing differential responses to various cuticular extracts and individual CHCs. Additionally, we identified HsOr263 as a receptor for a fertility signal produced by H. saltator reproductives. Taken together, the collective HsOr response profiles provide insight into the combinatorial coding of CHCs required for kin recognition. Our findings broadly implicate odorant receptors as the molecular basis of CHC detection in ants, with this receptor family likely being central to CHC-based identification in other eusocial insects.

How to sequence an ant? A Comparative analysis of approaches to sequencing and assembly of hymenopteran genomes

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This study is presented with two purposes in mind. First and foremost as a comparative study of our approach to the sequencing and assembly of hymenopteran genomes. Second to introduce a first draft for the genomic sequence of the desert ant Cataglyphis drusus. This new genomic resource will form the basis for future research, determining the genetic architecture of cuticular hydrocarbons synthesis, a key ingredient for nestmate recognition in Cataglyphis ants.

Our approach is unique because the core sequence data for the genome and transcriptome assemblies derived from a single haploid male. The use of haploid males as opposed to diploid workers/queens, and the extraction of both RNA and DNA from the same male individual simplify the assembly process thanks to the lack of polymorphism. Moreover, pairing the source of the sequenced DNA and RNA, provides more confidence in transcript-to-genome alignment, and eases the annotation of gene structure in terms of exon/intron boundaries. Alongside this, a DNA pool extracted from workers collected from a single nest provides the source for construction of large scaffolds and eventually improves the contiguity of the assembly. An additional RNA pool composed of a mixture of different castes and life stages from multiple nests is produced to assist with transcriptome construction.

To evaluate our approach we compare our assembled genome to other hymenopteran genomes assemblies, including those initially based on a single haploid male (e.g. Solenopsis invicta) and others that were not. Our novel approach incorporates the unique advantages for genomics in hymenoptera, namely haplodiploidy.

The role of ants in a natural insect-plant multitrophic system: first field survey in Italy on common vetch plants (*Vicia sativa*)

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Interspecific cooperation is a driving force in the evolution of many organisms. For this reason, symbioses are receiving increasing attention from different disciplines, not only for the evolutionary implications but also for their ecological impact. In this context, interactions involving ants and plants constitute textbook examples in animal-plant mutualistic relationships. In most cases, this system is characterized by trophobiosis in which the plants provide food to the ants that in turn offer protection from predators and competitors. In Italy, despite the rich ant-fauna and high plant diversity this phenomenon has been poorly studied. The present work is the first extensive survey conducted in Italy on interactions involving ants, other arthropods and plants in natural areas located near deciduous woods in the North of Tuscany. As plant model system we used the common vetch, *Vicia sativa* (Fam. Fabaceae) that produce extrafloral nectars on which ants feed. The observations allowed us to: a) make a characterization of the ant-fauna present on the plants; b) quantify the extent of these interactions; c) study the impact of ants on the presence and abundance of other arthropods on the plants. In this way, we clarified several eco-ethological aspects of these relationships, recorded the effective defensive role of the ants on plants and, therefore, their influence on the multitrophic network involving insects and plants in the studied environments.

Aphid-tending ants adapt their agonistic responses to aphidiphagous hoverflies

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Aphid-tending ants that feed on honeydew excreted by aphids should have evolved strategies against aphidophagous insects and should tune their aggressive behavior according to the level of danger for their trophobionts. Here we show that *Lasius niger* ants faced with *Episyrphus balteatus* hoverflies were less aggressive against early syrphid instars (eggs, L1 and L2) than against the voracious 3rd larval instar (L3), which was intensively bitten by ant workers. As a response, L3 tried to escape from ants by releasing a sticky - and possibly toxic - secretion over biting ants that behaved abnormally and were life-endangered. In a standardized system involving all the interacting agents i.e. the host plant, the aphid, the tending ant and the hoverfly instars, we found out that early *E. balteatus* instars induced no change in the number of foraging ants that often succeeded in removing the aphid predator out from the host plant. By contrast, L3 syrphids triggered an increase of ant patrollers at a local scale and proved to be quite efficient at resisting to removal attempts by ants. While supporting the assumption that ants tune their defensive response depending on the aphidophagous predator, our results also showed the lack of efficient protection of their trophobionts from the most voracious late syrphid instar.

Macronutrients in a termite species: Intake and impact on longevity

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Termites are an ecologically successful group, which can make up the major part of the insect biomass in soils, and as such they have a strong impact on their ecosystems. In that aspect and as they can be a threat to crop, tree plantations and houses, their highly specific digestion mechanisms had been studied for decades. Surprisingly little is known however as regards to their 8. Nutritional ecology. Here we present a new method to study termite intake for macronutrients (carbohydrate, protein and fat) based on the Geometric Framework for nutrition. This method relies on dry powder based diets and allows precise measurements of the termites' food intake, by giving them access to and weighing their consumption of 3 different diets: one rich in carbohydrate (cellulose), one rich in protein (whey protein), one rich in fat (phytosterols). I will present results gathered with this method in the Australian termite Nasutitermes exitiosus. Their intake in macronutrient was measured for the first time, and in two different conditions, to investigate the influence of the caste ratio: we used either groups of 70 workers and 30 soldiers, or the opposite. To further study the impact of macronutrient balance on termites, we investigated the effect of being constrained to a specific ratio of macronutrient on the individuals' longevity. These results are the first of their kind in termites, and the method opens promising doors to improve our understanding of termite nutrition and ecology.

Ecological relationships between social insects and *Saccharomyces* yeasts

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Nowadays, the presence of *Saccharomyces cerevisiae* has been assessed in both wild and human-related environments. Social wasps have been shown to maintain and vector *S. cerevisiae* among different environments and represent an important spring of yeast variability. The availability of strains isolated from wasp intestines thus represents a striking opportunity to assess if these strains are characterized by peculiar traits. In a recent research we analyzed strains isolated from social wasps' intestine and compared them with strains isolated from other sources, all collected in a restricted geographic area. We are now investigating if *cerevisiae* strains isolated from wasps have peculiar metabolic traits favoring production of volatile molecules attracting insects. We thus measured volatile metabolites during grape must fermentation for a set of wasp, grape and wine strains. Wasp strains, in addition to represent a wide range of *S. cerevisiae* genotypes, also represent large part of the phenotypes characterizing the sympatric set of yeast strains while their higher production of ethyl acetate could explain an improved ability to attract insects. We are currently performing a wider survey of other social insects, including ants and bees, to establish possible ecological and biological relationships with *Saccharomyces* yeasts.

Behaviour of single young honeybees and groups of young honeybees in a temperature gradient.

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It is a known fact that young honeybees have a temperature preferendum near 36° C. They show thermotaxis and locate themselves at their preferred temperature. Experiments were conducted to investigate the temperature preferendum of single, young honeybees in a temperature organ. Comparable experiments were conducted by Heran, 1951 with groups of honeybees. Surprisingly Heran never investigated single bees therefor we cannot exclude a social component of this thermotactic behaviour. In experiments in a circular arena with a flat gradient we found a strong, social component in the thermotactic behaviour of young honeybees. This behavior is well described by the BEECLUST algorithm. To exclude the social influence in this experiment we conducted trials with single bees (n=98). Only a minority of single honeybees are able to locate themselves at the optimum when introduced in a flat gradient and a round arena. We wanted to know how single bees behave in a rather steep gradient in a temperature organ (15-50° C). We placed bees in a temperature organ and recorded their position every minute, experiments lasted for 30 minutes. The mean temperature of the preferendum of young, single bees in our experiments was at 33° C, in contrast to Heran who found a mean temperature for groups of bees at 36° C. We could experimentally show that single, young honeybees in a temperature organ show a different thermotactic behaviour than groups of bees.

Are vegetated wood ant mounds at a disadvantage?

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Wood ants (*Formica rufa* group) typically built dome-shaped nest mounds that are covered with surrounding plant material such as pine needles, grass and twigs. Generally they keep their nests free of living vegetation as this allows more sunlight on to warm the nests. The ants regulate nest temperature so that from early spring to late autumn a nest is warmer inside than the air temperature outside. Temperature is important for faster brood development. Rarely, nests of *Formica aquilonia* are found completely covered with living vegetation and previous studies have concluded this is disadvantageous, with vegetation then manually removed as a conservation management tool. *F. aquilonia* appears on the Scottish Biodiversity List and is a species of conservation concern globally. We therefore sought to assess potential advantages or disadvantages of vegetated nests using 21 nests in Birse forest, northeast Scotland. We compared nest characteristics (height, diameter and volume), ant activity levels, and measured surface and internal temperature from vegetated (60-100% covered) and non-vegetated (<25% covered) nests. We also attempted to capture predation on the nests by birds/mammals using camera traps. Vegetated nests were taller and larger in volume than non-vegetated nests. Preliminary results show temperature differences but these are due to the location of the nests rather than features of the nests themselves. This suggests that these vegetated mounds are situated in a more suitable microclimate enabling them to grown larger and survive longer. We conclude that these nests appear not to be disadvantaged by the vegetation covering the mounds.

Sociogenomics of maternal care and mother offspring interaction in the earwig (*Forficula auricularia*)

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Cooperation and conflict are ubiquitous, especially in animals with parental care where parents and offspring reciprocally influence each other's behavior. Models have predicted the selection for parent-offspring co-adaptation through the co-regulation of maternal and offspring genes. However the genomic mechanisms of parent-offspring interactions were poorly understood. We used the sub-social European earwig as model organism, manipulated mothers and their offspring's behavioral interaction and sequenced 84 transcriptome libraries from five tissues of three experimental groups. We found two genes co-regulated in mothers and offspring, one involved in a chemosensory pathway and the other in a synaptic signaling pathway. Their RNAi knock-down resulted in behavioral and developmental change in mothers and offspring, which is consistent with between-castes gene expression variation in eusocial termites. Our results also indicate that a mutual rewarding system is activated for the maintenance of the social-bond between mother and offspring. Such a mechanism may be essential for the evolution of parental care and family living. Furthermore for the first time in a maternal care system, we found a eusocial-specific endocrine pathway (including juvenile hormone and vitellogenine), similar to the queen of honey bee. This supports the theory that eusociality was probably originated from the evolution of parental care.

Conditions allowing for the coexistence of Independent vs. Dependent Colony Foundation

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Reproductive investment must balance the trade-off between offspring size and number (few large vs. many small offspring). While larger offspring can be more competitive, this often comes at a cost to their colonisation ability. This competition-colonisation trade-off can allow alternative strategies to coexist in the same environment. Several studies also stressed the importance of the spatial structure of the environment in supporting strategies coexistence. Social insects are ideal models and we used an agent-based model that explicitly considers space to investigate when two ant species identical in every respect except their mode of colony foundation (Dependent vs. Independent Colony Foundation) can coexist. DCF emphasises competitiveness (large offspring and low dispersal mortality) whereas ICF emphasises colonisation ability (long dispersal and high offspring number). Our simulations show that ICF outperforms DCF in environments subject to high temporal or spatial heterogeneity, when colonies mature and reproduce late, or when colonies invest heavily in reproduction. On the other hand, DCF dominates in low-disturbance and high resource environments, or when maintenance costs are low. Varying life-history parameters and environmental parameters had a marked influence on coexistence conditions and yielded evolutionary stable strategies with either DCF or ICF totally outcompeting the other strategy. Nonetheless, ICF and DCF coexist over a wide range of parameter values. This can be explained by the competition-colonisation trade-off, and by habitat heterogeneity that also favours coexistence.

Phylogenetic and phenotypic divergence in the Australian Amitermes Group

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Members of the Australian Amitermes Group (AAG, mainly *Amitermes* and *Drepanotermes*) exhibit traits unusual in the termite world, including foraging in the open, harvesting and storage of foodstuffs, multiple active reproductives of both sexes within a single nest, and nest parasitism (inquilism), alongside species with more common foraging, nesting, and reproductive strategies. The ~100 described species in this group diverged and evolved these disparate traits during a period of dramatic climate change occurring since Australia's separation from Antarctica and South America (55 MYA) through the end of the Pleistocene (1.6 MYA). Here I present initial results from a preliminary data set of 10 AAG species, demonstrating the application of ecological biogeography techniques to this group. I would love to say something specific about the results here, but I'm writing this at the beginning of April and we are still doing the work. Future work incorporating all AAG species will help us understand evolutionary pathways to the modern trait constellations and open the potential for predicting how traits and ecological function may respond to current climate change.

Ant community changes under increasing land-use intensity in temperate grassland

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Ants are an important functional group of temperate grassland ecosystems. Land-use intensification has been shown to have a negative influence on ant diversity. However the mechanisms leading to these effects are not well understood and may affect different ant species differently, as ant species may differ in trophic niche. Predatory ant species or those with a broader nutritional niche are less affected as prey items are still available in intensively used plots while trophobionts strongly decrease, potentially limiting the availability of carbohydrates.

To investigate the changes in ant communities, we sampled ants in 140 temperate grassland plots (50x50m) in three regions in Germany within the Biodiversity Exploratories using pitfall traps (12 pitfall traps per plot) and hand-sampling. The grasslands are used as meadows or pastures at different intensities.

We found that ant species richness decreased significantly with increasing land-use. This was not only the result of losing particular ant species, but also resulted from a turnover in occurring species. As a consequence, functional diversity of ant communities decreased significantly with larger and subterranean species being absent at higher land-use intensity. Apart from the decreasing number of species we also found a reduced colony density and smaller nest-mound sizes with higher land-use intensity. This indicates that some grassland ant species seem to be less affected by land-use intensification, which might relate to the trophic niche of the ant species in question. To determine the niche we are conducting stable isotope analysis for the ant species present over the land-use intensity gradient.

Social polymorphism or cryptic speciation in the desert ant *Cataglyphis*

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Species in the genus *Cataglyphis* show a remarkable diversity in social structures. Using genetic, and chemical analyses we characterized populations of the *bicolor* group in Israel. A North-South transect along the coastal plain revealed a succession of three *bicolor* sub-groups, tentatively assigned as *C. drusus, C. niger*, and *C. savignyi,* respectively. Species delimitation was further investigated using cuticular hydrocarbons chemistry and a combined set of microsatellites, mitochondrial and nuclear genetic markers. Our results show that mitochondrial markers vary along a north-south gradient. The Northern populations (*C. drusus*) differ from the Southern populations (*C. savignyi*), whereas a mixture of the mitochondrial haplotypes exists in the central coastal plains of Israel (*C. niger*). Interestingly, nuclear sequences do not delineate the groups highlighted by mitochondrial markers and do not provide support for the distinctiveness of these putative species.

The social structures of selected populations within these sub-groups were also investigated. The Southern population (C. savignyi?) is monogyne and monodomous (each colony has a single nest), while the Northern population (*C. drusus?*) is monogyne and polydomous (multiple nests per colony). The central population (*C. niger*) is the most striking. It is highly polygyne and exhibit a transition from polydomy to supercolony. Thus along a relatively small distance in the Israeli Coastal plain there is a wide gradient of social structures. The question of whether these mitochondrial haplotype differences represent cryptic species, each of which developed a disparate social structure, or whether it represent a single species that adaptively evolved different social structures remains open.

Heat resistance of the thermophilic ant *Aphaenogaster iberica* along an altitudinal gradient in Southern Spain

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Local adaptation is a powerful process to explain how species with wide geographic distribution tolerate contrasting environmental conditions. Hence, natural selection acting on discrete populations can promote the fixation of different adaptive traits. In ants, as in most insects, traits involved in resistance to local climatic conditions are expected to be under strong selection since important functions like development, growth and activity are temperature-dependent. Here, we present the results of a study conducted on the thermophilic ant *Aphaenogaster iberica* along an altitudinal gradient, from 100 to 2000 meters, in Sierra Nevada (Southern Spain). We combined behavioural, morphological, physiological and molecular analyses to determine between-populations differences in traits involved in temperature and dryness resistance. Field data show that populations located at mid-elevation, where ground surface temperature can rise up to 70ºC, have their higher daily activity at a warmer temperature than populations of low and high elevation (and cooler temperatures). In laboratory conditions, workers of mid-elevation populations survive longer when they are exposed to heat which is supported by measures of the individual activity (a proxy of the metabolic rate) as a function of temperature. In contrast, there was no difference between populations regarding resistance to cold and dryness. Measures of gene flow between populations are also conducted using 10 polymorphic microsatellite markers. Results suggest limited gene flow between populations may contribute to the above-mentioned phenotypic differences.

Drivers of pine tree invertebrate communities: tree chemistry or wood ants?

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Wood ants affect the abundance and distribution of many other organisms in boreal forests, both positively (e.g. the mutualism with aphids), or negatively as predators. Both sucking (e.g. aphids) and chewing invertebrates are also affected by the chemical composition of host trees, which in conifers comprises large amounts of volatile compounds such as monoterpenes, many of which act as repellents or toxins. Within pinewoods, there is considerable genetically-based chemotypic differentiation between individual trees based upon their monoterpenes (e.g. some contain 3-carene in their needles whilst others contain none). We investigated the relative importance of woods ants (*Formica* spp.) versus tree chemotype in shaping Scots pine (*Pinus sylvestris*) crown invertebrate community using two approaches. Firstly, we conducted a two-year survey of ant activity and the invertebrate community on trees of known chemotype in the Ballochbuie native pinewood in northeast Scotland. Trees with zero 3-carene contained larger populations of crown (non-aphid) invertebrates than trees with 3-carene. However, this effect was only evident when the tree was not used by foraging wood ants. To experimentally distinguish the effects of ants from tree chemistry on crown invertebrate communities we transplanted 168 saplings of known chemotype into ant and ant-free zones of the forest. Over three years, we monitored crown colonisation by invertebrates and found that wood ants, rather than chemotype, were the strongest driver in shaping non-aphid invertebrate crown communities. As ecosystem engineers, wood ants have strong effects on biodiversity and forest ecosystem function, which should be considered when planning and planting new forests.

Vibroacoustic communication in *Crematogaster scutellaris* ant (Oliver 1792)

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Communication is a crucial feature for ant colonies, which are formed by thousands of interacting workers. Although chemical signals remain the main modality of intracolony communication, several ant species can exchange information also through substrate-borne vibrations. In many ant species vibrational signals are produced by stridulation, that occurs in many different behavioural contexts according to the species, their social organization and environment. We studied the stridulatory behaviour in Crematogaster scutellaris, a dominant tree-nesting myrmicine Mediterranean ant, in different behavioural situations, such as routine daily activity inside the nest, food retrieval by workers and a simulated predator attack. Recordings were carried out using a laser vibrometer, and the signals emitted by three different castes (queens, workers and males) were analysed and compared. Playback tests were performed with an electromagnetic shaker, to investigate the ability of vibroacoustic signals to trigger a response in conspecifics. All castes were able to produce long sequences of signals formed by disyllabic chirps. The signal is distinctive of each caste, considering temporal (e.g. the number of bursts, chirps duration) and spectral parameters. Worker signal has higher dominant frequency (above 1000 Hz) and lower intensity compared to reproductive individual signal (dominant frequency below 600 Hz). The function of these signals probably changes according to the context: whenever an individual was trapped, stridulation occurred, thus suggesting a deterrent function, but workers stridulated also when a drop of honey was offered to them to recruit nest mates. On the contrary, no vibrational signals were ever recorded during normal nest activity.

Formicidae stratification in an agroforestry system in Brazil and its importance in plantation management

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Together like some coffee crops, the shaded cacao plantation is considered the agricultural environments that best preserves the native animal diversity. In the cocoa agroforestry existing in southeast of the state of Bahia, Brazil, the vegetation offers a close structure to that of native rainforest: shading trees constitute the canopy, they are covered by a range of epiphytes. In the canopy, bromeliads maintain water reserves and provide shelter opportunities to arboreal ants besides attracting potential preys. The shrubs are almost exclusively cacao trees. On the floor, the undergrowth covers a thick leaf litter composed primarily of rotting vegetal material fallen on the ground and covering the mineral soil. Vertical physical gradients that more contribute to stratifying the fauna are light incidence, thermal amplitude and humidity saturation. All these conditions contribute to promote fauna stratification: polydomous species of the genera Azteca or Cephalotes or occupants of hollow branches such as Pseudomyrmex spp. nesting in the canopy. On the shrubs or associated with epiphytes, there are numerous species of Neoponera or Dolichoderinae, Formicidae or Myrmicinae representatives, well adapted to arboreal life with a generalist behavior, while others of the same groups are living in the leaf litter on the ground. It is yet in the litter or in the soil that Cerapachyinae, Dorylinae, Ectatomminae or Ponerinae most specialized ants inhabit or forage. With about 180 species per hectare, the ant community living in cacao plantations is the most effective regulator of insects potentially harmful to this economic activity.

Investigating key traits for AQS emergence in Termitinae

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In termites, the traditional view of reproductive systems has been recently challenged by multiple records of sexual reproduction systematically combined with parthenogenesis. Using this reproductive strategy, called Asexual Queen Succession (AQS), the founding primary queen is replaced by numerous parthenogenetically produced daughters (neotenics) that mate with the primary queen's former partner. AQS is now firmly demonstrated in five species (three *Reticulitermes* species, *Embiratermes neotenicus* and *Cavitermes tuberosus*) from two families, in various ecological contexts, and in which parthenogenesis occurs through different cytological mechanisms. This diversity suggests that AQS evolved independently and multiple times in termites.

We compared two Termitinae, *C. tuberosus* and a closely related sympatric species from an undescribed sister genus for which we also report the occurrence of AQS. Both species share similar life history traits: they are tropical soil-feeding termites, and produce throughout the year pre-neotenic female nymphs that are entirely homozygous, indicating parthenogenesis through gamete duplication.

However, fundamental differences occur between these two species in the colony life cycle. Notably, the replacement of the primary queen by neotenics seems more frequent in *C. tuberosus*. Moreover, contrary to *C. tuberosus*, primary queens of the undescribed species reach a high level of physogastry. Both traits suggest that primary queens of the undescribed species live longer than those of *C. tuberosus*, and that AQS may not occur systematically in the life cycle of the former species. Our comparative analysis sheds light on essential preadaptations for AQS emergence in Termitinae and other families.

The Genetic Basis for Slavemaker Raiding and Host Defensive Behavior in *Temnothorax* Ants.

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Ants within the genus Temnothorax possess a number of noteworthy traits ideal for the elucidation of behavior and co-evolution within social Hymenopera. Of particular interest is the observation that social slavery has arisen, independently, multiple times within Temnothorax. Recent phylogenies suggest that slavemakers tend to be closely-related to their primary host, and that they co-evolve with this host species through reciprocal adaptations. Thus, the aim of this project is to elucidate the basis for the evolution of behavior in social Hymenoptera through the examination of the genetic components responsible for slavemaker raiding behavior and, reciprocally, host defensive behavior. To investigate this, we utilized a gene expression approach, precluded by the assembly of unique transcriptomes from behavior-specific RNA-Seq data. We have found that slavemaking species possess a higher number of significantly differentially-expressed genes, both during 'active' (raiding) and 'passive' (inactive) behavioral states; when compared to host species (defensive vs. non-defensive behavior, respectively). Similarly, our findings indicate that slavemakers possess a greater number of unique, species-specific over-expressed genes during their 'active' state than hosts. For both slavemakers and hosts, patterns of similar gene expression between species closely follow phylogenetic lines. Functionally, we find a number of behavior, transcription-factor, immunity, and circadian rhythm genes over-expressed within both host and slavemaker species. Finally, through the use of RNAi-mediated gene knock-down, we experimentally determined the function of select over-expressed candidate genes within Temnothorax. One gene of interest, Drosophila-described painless, we have found to likely be involved in promoting 'risky' behavior in slavemaker raiders.

Zero waste and no known diseases in fungus farming termites

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Thirty million years ago, the ancestors of the termite sub-family Macrotermitinae and the basidiomycete *Termitomyces* joined forces in what became one of the most sophisticated plant-biomass decomposition symbioses on Earth. Through intricate interactions between an external fungus garden and the termite host and its accompanying gut microbes, this symbiosis manages to fully decompose nearly any plant polysaccharides and has no apparent problems with infectious diseases. 16S rRNA 454 pyro-sequencing portraying community compositions of Macrotermitinae core gut microbiotas and Illumina HiSeq metagenome sequencing, show that the innovation of fungiculture induced a compositional and functional shift in the Macrotermitinae gut microbiota. Gut bacteria contribute fungilytic enzymes and enzymes needed for final plant decomposition after *Termitomyces* degrades the complex carbohydrates. This implies that the shift in gut microbial capacity after *Termitomyces* domestication involved the evolution of complementary division of symbiont labour. A second gut passage of the fungus comb thereby ensures that all polysaccharides are utilized. Obligate gut passage of the plant substrate may also assure that potential antagonists are efficiently suppressed before entering termite nests, and I will end with a discussion of evidence for this hypothesis.

Fine-scale population genetic structure in the hybridogenetic ant *Cataglyphis mauritanica*

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Some species of *Cataglyphis* desert ants have evolved a hybridogenetic mode of reproduction. Populations are characterized by the co-existence of two genetic lineages. Queens typically mate with a male of the alternative lineage and use its sperm to produce hybrid workers. In contrast, new queens and males are produced asexually by parthenogenesis. As a consequence, the two lineages are maintained genetically distinct over generations despite constant hybridization for worker production. This reproductive system is expected to have strong consequences on population structure because both lineages must coexist and interbred.

We performed a detailed population structure analysis of the hybridogenetic species *Cataglyphis mauritanica.* Our sample consisted in 312 colonies, covering 55 ha in the Ifrane National Park, Morocco. Our results show strong sex-biased dispersal. We found a genetic structure for queens, consistent with their parthenogenetic production and short-distance dispersal strategy. In line with this, the population consists on a mosaic of patches within which queens are clonal, hence, belong to a single genetic lineage. We estimated the dispersal distance of males by inferring their patch of origin based on the genotype of the sperm stored in the spermatheca of queens. Males disperse at a greater distance than females, and mate in patches from the alternative lineage. However, they display a non-random spatial genetic structure, indicating that males experience dispersal limitation at the scale of the population.

Overall, this study shows how social hybridogenesis affects gene flows within populations and how this results in a unique population structure in *Cataglyphis* ants.

The importance of host phylogeny and geography on the microbiome of wild bees

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Social corbiculate bees such as honey bees and bumble bees maintain a beneficial core microbiome which is absent in wild bees. Here we combine new and previously published data to compare the microbiomes of 10 wild bee species from the genera *Augochlora*, *Megalopta*, *Halictus*, *Megachile*, *Osmia*, *Lithurgus* and *Ceratina*. Our study will test for the existence of a wild bee core microbiome. We will additionally test for associations between the microbiome structure and host phylogeny, geography, and level of eusociality. We determine that microbiome composition is strongly correlated to host geography and provide evidence that wild bees may obtain much of their microbial community via environmental transmission from flowers. Despite the importance of environmental transmission, bee phylogeny was also correlated with the gut microbial community. The consequences of this enviro-phylo associated microbiota are discussed in relation to maintaining the health and avoiding dysbiosis of fragmented or introduced populations of wild bees.

Phylogenomics controlling for base compositional bias reveals a single origin of eusociality in corbiculate bees

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As increasingly large molecular data sets are collected for phylogenomics, the conflicting phylogenetic signal among gene trees poses challenges to resolve some difficult nodes of the Tree of Life. Among these nodes, the phylogenetic position of the honeybees (Apini) within the corbiculate bee group remains controversial, despite its considerable importance for understanding the emergence and maintenance of eusociality. Here we show that this controversy stems in part from pervasive phylogenetic conflicts among GC-rich gene trees. GC-rich genes typically have a high nucleotidic heterogeneity among species, which can induce topological conflicts among gene trees. When retaining only the most GC-homogeneous genes or using a non-homogeneous model of sequence evolution, our analyses reveal a monophyletic group of the three lineages with a eusocial lifestyle (honeybees, bumblebees and stingless bees). These phylogenetic relationships strongly suggest a single origin of eusociality in the corbiculate bees, with no reversal to solitary living in this group. To accurately reconstruct other important evolutionary steps across the Tree of Life, we suggest removing GC-rich and GC-heterogeneous genes from large phylogenomic datasets. Interpreted as a consequence of genome-wide variations in recombination rates, this GC-effect can affect all taxa featuring GC-biased gene conversion, which is common in eukaryotes.

The genomic basis of slavemaker evolution in the ant genus *Temnothorax*

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Social parasitism is a special form of parasitism in which the parasite exploits the social behaviour of its host. Slavery evolved repeatedly in the ant genus Temnothorax, in which slavemakers set out on raiding parties, to steal the brood of their hosts. To investigate the genomic basis of slavemaker evolution, we made use of transcriptome sequence information of three slavemaker and three host species to a) construct a phylogeny based on 5199 loci, and b) identify genes with signatures of positive, as well as relaxed selection. We expected genes to be under selection that are important in host-parasite coevolution such as those involved in recognition processes. Indeed, we found genes involved in cuticular hydrocarbon synthesis and olfactory receptors under positive selection, yet others were under relaxed selection. This pattern fits a co-evolutionary arms race scenario, in which co-adaptation leads to selection of certain genes, whereas others become insignificant. Slavemaker workers are highly specialized and show a reduced behavioural repertoire in comparison to host workers. Hence we predicted genes involved in typical worker behaviour to be under relaxed selection in slavemakers. Indeed, we found 56 genes with various functions under relaxed selection in slavemakers, and none in hosts. The identification of selective forces shaping the slavemaker versus host lifestyle also allowed us to investigate whether parallelism increases from gene to the molecular pathway level. Our species comparisons identified different genes, functions and pathways to be under selection, pointing to convergent rather than parallel trajectories in the evolution of the slavemaker lifestyle.

The adaptive significance of fungus-growing termite microbiotas to the evolution of fungiculture

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A major evolutionary transition took place in the higher termites 30 MYA, when the subfamily Macrotermitinae domesticated a plant-degrading *Termitomyces* fungus crop. Termite workers forage for plant material and mix it with fungal spores in their guts to build a faecal substrate (the fungus comb) where *Termitomyces* grows. Soldiers defend the colony and mainly ingest fungal biomass. A core community of 42 gut bacteria are shared among macrotermitine species, and fungal domestication influenced gut microbiotas to become different from other termites, but similar to the ancestral omnivorous cockroaches. Using 16S rRNA amplicon sequencing, we here explored whether gut microbiota compositions are affected by caste roles, and whether fungus combs (due to their origins as termite faeces) aintain stable microbiotas. Analyses of 1360 termites from workers and soldiers from three termite species, from four sites in South Africa in 2013-2015, confirmed that gut microbiotas are taxonomically and structurally stable over space and time. These analyses, however, elucidated differences in relative abundances of a subset of bacterial genera between castes, indicating that the division of labour extends to gut microbiota compositions. By characterising fungus comb bacterial communities, we show that gut bacterial taxa are, present in combs; however, comb communities are more variable over time than guts, due to bacterial influx from the environment. Collectively, our findings show that Macrotermitinae adopted not only their fungal ectosymbiont as a crop and external digestive partner, but also maintain specific sets of bacteria adapted to functional differences between castes and between gut and comb environments

Evolutionary dynamics of social hybridogenesis in *Cataglyphis* desert ants

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Some species of *Cataglyphis* desert ants have evolved a hybridogenetic mode of reproduction. In each population, two divergent genetic lineages co-occur as a complementary pair. Queens typically mate with a male of the alternative lineage; non-reproductive offspring (workers) are hybrids of the two lineages, whereas sexual offspring (males and new queens) are produced by parthenogenesis and belong to the mother queen lineage.

We explored the distribution of hybridogenesis across populations of two species, *C. velox* and *C. humeya,* from the Iberian Peninsula. We sampled 68 localities covering the whole distribution range of both species. In each population we examined the origin, sexual or parthenogenetic, of the different castes.

Our results show both inter and intraspecific variations in the mode of reproduction: whereas most populations of *C. velox* display social hybridogenesis, all populations of *C. humeya* and some remote populations of *C. velox* use sexual reproduction. Using a combination of microsatellite loci and RAD-seq markers, we determined the evolutionary dynamics of hybridogenesis from the phylogeographic relationship between hybridogenetic and non-hybridogenetic populations.

Genetic analysis reveals multiple introduction events of the Balkanic *Reticulitermes urbis (*Blattodea,Termitoidae, Rhinotermitidae) in Italy and France.

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Biological invasions are a key factor in the ecological changes, and social insects are among the most successful invasive animals. Phylogeography and population genetics can provide detailed information on the introduction routes and invasion biology. *Reticulitermes urbis* is a subterranean termite native of Balkan Peninsula and introduced in Southern France and Eastern Italy. Introductions in these countries probably resulted from anthropic activities and invasive populations currently live in both forests and urban areas. To identify the source population and to infer the minimum number of introductions, we analyzed mitochondrial (COII) and nuclear (6 microsatellites) loci on 7 native and 39 introduced colonies (15 from France; 24 from Italy). Mitochondrial analysis confirmed the presence of two major lineages in which native and introduced populations cluster together, irrespective of the sampling area (native or invasive range). Microsatellite loci analysis identified two genetic clusters, each including individuals from both native and introduced samples. Moreover the analysis of molecular variance evidenced very low genetic differentiation between the three considered areas (native, French or Italian range). Finally, data showed that introduced populations are less variable in both mitochondrial and nuclear markers, suggesting that introductions might have induced a loss of genetic diversity. On the whole, results supported multiple introductions into the two invasive ranges of France and Italy. This is consistent with links between native and invasive areas protracted in time, as expected in cases of human trades routes. Historical data of human settlement and kingdoms might provide explanations for *R. urbis* modern distribution.

New case of asexual queen succession and its implications for understanding the significance and evolution of parthenogenetic reproductive strategies in termites

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Over the past few years, a number of studies on the genetics of colonies and populations uncovered a remarkable diversity and versatility of reproductive and dispersal strategies of termites. Among them, the asexual queen succession (AQS), combining the sexual process for the production of sterile helpers and dispersing reproductives with thelytokous parthenogenesis for the production of non-dispersing neotenic queens, has recently been identified in several species. While the resulting breeding systems share many similarities, the phylogenetic distribution of AQS and functional differences in the parthenogenetic process clearly suggest multiple independent origins of this outstanding mixed reproduction in different lineages.
We will present a new case of AQS in the higher termite Silvestritermes minutus (Termitidae: Syntermitinae). Beside the genetic structure of colonies, we will show our observations on the genetic structuration at the population level, dynamics of dispersal and a reconstruction of the colony life cycle from its foundation by a pair of primary reproductives, through queen replacement by multiple parthenogens to the single major dispersal event and colony decline. We will discuss our findings on S. minutus in the comparative context of the other cases of AQS in an attempt to highlight similarities and differences and underline the versatility of the role of AQS in the life histories of different species. Last but not least, based on the current knowledge on the phylogenetic and geographical distribution of confirmed AQS occurrences in Termitidae, we will predict that numerous cases of AQS are yet to be discovered.

Phylogeography of European dry-wood dwelling termites of the genus *Kalotermes* (Isoptera, Kalotermitidae).

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The only native European dry-wood termites belong to the genus Kalotermes. Until recently, these termites were thought to belong to a single species, Kalotermes flavicollis. However, recent studies described two new species: K. italicus (Italy) and K. phoenicae (Cyprus, Middle-East). Moreover, molecular phylogenetic studies revealed divergent K. flavicollis sublineages in Corse-Sardinia and Southern France. As such lineages have been shown to frequently merge in same colonies, it is important to better understand the evolution of this genus in Europe. To widen the analyses on Kalotermes phylogeography, 22 colonies collected in 11 European localities were analyzed together with those obtained from previous studies, sequencing 912 bp of the mitochondrial genome (COI/tRNA-Leu/COII) in two individuals/colony. Phylogenetic analyses indicate that (i) K. italicus spreads from Central Italy to South France, (ii) the Corse-Sardinian lineage can also be found in the Italian peninsula, and (iii) the Southern France lineage extends to the Iberian peninsula. A time-calibrated phylogeny, computed on two mitochondrial markers (COI/tRNA-Leu/COII; 16S) on representative samples, showed that (i) extant European Kalotermes started differentiating ~32 Myr ago, with the separation of K. phoenicae; (ii) K. italicus and K. flavicollis split ~15 Myr ago; (iii) K. flavicollis sublineages diverged during and after the Messinian salinity crisis, ~6 Myr ago. Another European termite genus, Reticulitermes, comprises in the same area seven species/subspecies and all taxa showed marked intraspecific differentiation. Interestingly, recent analyses indicated that Reticulitermes differentiated in half time with respect to Kalotermes. The different nesting ecology and/or habitat constraints may explain this significant difference.

Diversity and composition of the hindgut microbial communities in lower termites

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Nutritional mutualistic symbioses are mutually beneficial associations for metabolic exchange that occur between a host and its symbiotic microorganisms. Termites are wood-feeding social insects that exhibit complex nutritional mutualistic symbioses with an assemblage of gut microorganisms including prokaryotes (archaea and bacteria) and flagellated eukaryotes (Parabasalid and Oxymonad protists). In termites, microbial communities have a key role for lignocellulose degradation. On the other hand, microbial communities depend on termites for anaerobic habitats and nutrient supply. The role and interactions of multiple symbionts have relevant evolutionary implications for our understanding of the dynamics of host-symbiont nutritional symbiosis. For instance, different microbial taxa may perform complementary or even synergistic functions for their host. The first step to better understand these complex interactions is to identify the different symbiont species that are present in termite's guts. We analyzed bacterial communities from two foraging species of the genus *Reticulitermes* (Rhinotermitidae) *R. flavipes* and *R. grassei*, and from one wood dwelling termite of the genus *Kalotermes* (Kalotermitidae) *Kalotermes flavicollis*. Our study was based on a metabarcoding approach of the V3-V4 region of 16SrRNA.

Our results showed that these species exhibit associations with the following bacterial phyla: Bacteriodetes, Spirochaetae, Proteobacteria, Elusimicrobia and Firmicutes. Additionally, in terms of taxa composition, the wood dwelling *Kalotermes flavicollis* exhibited the lowest diversity when compared to *Reticulitermes* foraging species. Therefore, termite's lifestyle may shape microbial communities composition and abundance. Moreover, bacterial community's variation among wood-feeding termite taxa may reflect the different roles that nutritional mutualistic symbionts have in distinct termite species.

The genomes of the slavemaker ant *Temnothorax americanus* and its host *T. longispinosus*

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Slavemaker exploit the social behaviour of their often closely related host species, yet, their highly divergent life styles has favoured the evolution of distinct behavioural, chemical and life history strategies. While both slavemaker and host strategies have been extensively studied at the phenotypic level, the molecular mechanisms underlying trait divergence are unknown. We present the high quality draft genome sequences of the host ant *Temnothorax longispinosus*, and the congeneric slavemaker ant *T. americanus*. These two genomes allow us to investigate molecular traces of adaptation to the life style of slavemakers and hosts at the levels of gene regulation, coding sequences and gene losses, gains and rearrangements. Slavemakers frequently use chemical warfare to manipulate the behaviour of their host, and chemical mimicry and diversification of the cuticular hydrocarbon profile used in enemy recognition play an important role in the host-slavemaker co-evolutionary arms race. Hence, of particular interest are genes involved in odorant and gustatory receptors, desaturases and elongases. In addition, we focus on biochemical pathways involved in the reproductive division of labour because slavemaker workers, unlike host workers, frequently produce male offspring, even in the presence of a queen. These analyses provide a first insight into the molecular evolution of slavery and contribute to our understanding of this remarkable life history strategy.

Super-scaffolding the fire ant genome and detection of chromosomal rearrangements

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The fire ant Solenopsis invicta is an important global pest and an important model for social evolution. Its genome was assembled from short read sequences in 2010, and thus remains highly fragmented. This fragmentation has handicapped much molecular-genetic research.

We performed deep sequencing of fire ant males using the Bionano Genomics Irys nanochannel-based optical mapper to obtain nicking-enzyme recognition-site patterns for genomic fragments from 150,000 to 1,900,000 nucleotides long. Combining the resulting optical map with genetic maps and the old genome data leads to dramatic increase of genome quality by improved contiguity and elimination of assembly errors. We thus describe a novel approach for obtaining highly-contiguous genome assembly. Furthermore, performing comparisons between Irys maps generated from different individuals reveals several large chromosomal rearrangements in this species that may be involved in novel adpation.

Pollinator population genomics: Assessment of environmental stresses on bumblebee genetic diversity

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Insect pollination is an essential ecosystem service for the maintenance of ecologically and commercially important crop yields. Social bees, such as honeybees and bumblebees, contribute a substantial amount to pollination and therefore, recent global declines pose serious threats to both food security and ecosystem stability alike. Environmental threats, such as pesticide exposure and pathogens, have been highlighted as contributing factors to bee declines yet our understanding of the potential effects of such stresses at the molecular and genomic level of pollinator species is limited. To address this issue, we conducted a population genomic study on the buff-tailed bumblebee, *Bombus terrestris*, a common Eurasian species and key ecological pollinator. For this we performed whole genome sequencing of individual bumblebees collected from pesticide poor and pesticide heavy sites across the United Kingdom. This provides a comprehensive overview of genetic variation present across natural populations and allows for examination of molecular signatures of selection associated with exposure to pesticide. Furthermore, we characterized pathogens and parasites carried by each individual providing an insight into pathogen prevalence and diversity across bumblebee populations. Taken collectively, the results of this study provide a global insight into the genetic structure, health and pathogen loads of bumblebee males.

A genome-wide comparison between African highland and lowland *Apis mellifera* honeybees reveals ancient structural rearrangements associated with behavioral loci

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Large-scale structural rearrangements like duplications and inversions can have profound impact on genome function, generate novel phenotypes and serve as substrate for selection. We here present the first evidence of structural rearrangements associated with local adaptation in honeybees.

East Africa is a heterogeneous region spanning punctuated montane forests and widespread lowland savannahs. Honeybees inhabiting the highlands are classified as *A. m. monticola*. These bees are darker and exhibit different foraging and defense behaviors than the yellowish savannah bees. Despite its scattered distribution, it has been suggested that *monticola* represents a distinct subspecies. To test this hypothesis and to detect loci involved in altitude adaptation, we compared 39 genomes of honeybees from two Kenyan localities separated by 100km (Mau; Mt Kenya), each comprising bees from neighboring highland and lowland locations.

Using whole-genome sequencing, we detected 8 million SNPs. Genome-wide F_{ST} between any of the four groups is <0.06, indicating pervasive gene flow. Neighboring highland and lowland populations are the most similar, suggesting that *monticola* is not a distinct lineage. However, three chromosome blocks are extremely differentiated between all highland and all lowland bees. They contain most $F_{ST}>0.5$ SNPs (n=24,442) segregating between the two environmental regimes. Linkage between these SNPs is consistent with long (550kbp, 20kbp and 1,630kbp), unique and inverted highland haplotypes. Genetic divergence between haplotypes is 1.3-3.2%, suggesting they diverged before the origin of extant honeybee subspecies. They contain six of the seven honeybee octopamine receptors, which are important for learning and foraging, implicating these loci in high altitude adaptation.

Fine-scale population genomics and sex-biased dispersal in *F. selysi*

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In socially polymorphic ant species, the number of reproductive queens per colony varies, some colonies being headed by a single queen (monogynous) and other by multiple queens (polygynous). In Formica selysi, these alternative forms of social organization occur in sympatry and are associated with a supergene, which raises interesting questions about the maintenance of the polymorphism. In many ant species, monogynous and polygynous colonies differ in multiple components of their breeding systems, including mating system, queen dispersal and colony founding. Queens originating from monogynous colonies typically disperse on the wing and found colonies independently, while queens from polygynous colonies show restricted dispersal and found new colonies by budding. Here, we investigate if such differences occur between monogynous and polygynous colonies of Formica selysi, in order to evaluate if shifts in breeding systems restrict gene flow between social forms and contribute to maintain the social polymorphism. We used a large number of single nucleotide polymorphisms (SNPs) to study population genomic differentiation with unprecedented resolution. We reconstructed the genotypes of queens and their mates by analyzing workers sampled in monogynous colonies or coming from polygynous queens kept in isolation. In total, we genotyped 896 workers using single-end RAD-sequencing, which revealed over 1'000 SNPs. This powerful approach allows for detailed determination of spatial genetic structure and effective dispersal of each sex in Formica selysi, which will shed light on the mechanisms underlying the maintenance of the social polymorphism.

From gene list to gene network: recognizing the functional connections that regulate behavioural traits

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The study of social breeding systems has long incorporated molecular information. From the notion of 'genes for altruism' that underpins inclusive fitness theory to a now generation-long effort to uncover and interpret the molecular correlates to social variation, the field continues to assimilate ideas and tools from molecular biology. One common output from molecular studies in sociobiology is the ubiquitous gene list. These lists, which are readily generated from microarray, RNA-Seq or other molecular screens, can prioritize genes based on measured difference in expression, and the molecular function of the top-ranked genes can be inferred through homology-based annotations. This soft link between gene and gene function is unsatisfying however because the information is static, and does not in itself explain how genes interact with each other or how these interactions change in real-time with social or environmental circumstance. Here I promote a view common in systems biology where gene lists can be converted into gene networks that better describe the dynamics of gene regulatory effects on behaviour. I present one case study from honeybees, and show how analysis of gene network topology can re-prioritize genes based on connectivity, and reveal other functional connections that would not otherwise be apparent, including the presence of regulatory gene modules and clusters. Finally, I argue that because network analysis is not limited to 'genes' as nodes, it can potentially be applied across multiple levels of social organization within a single study system.

Biogeography of bacterial communities associated with gut ecosystem of termite *Odontotermes* sp.

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The Western Ghats of India comprises of natural forests that are teeming with various flora and fauna imparting significant biogeography. This region houses more than 95 species of termites with a predominance of *Odontotermes* genera (41%). These termite communities are regarded as the ecosystem modulators and their survival depends on diverse microbial community that subsist solely for the symbiotic digestion of lignocellulosic materials. Currently, the knowledge of gut bacterial flora limits to only cultivation dependent and hence culture independent high-throughput sequencing of the gut bacterial communities will underpin the complete phylotypes of uncultivated intestinal lineages. Therefore, our precedence is to analyze the patterns in the microbial composition of intraspecific bacterial communities (fore, mid and hindgut) of termite *Odontotermes* sp. through next generation sequencing (NGS). The DNA isolated from different portions of the gut were subjected to identification of phylotypes, by amplifying the V3-V4 hypervariable region of the 16S rRNA gene. The representative phylotypes were affiliated to 12 phylogenetic groups of Acidobacteria, Actinobacteria, Bacteroidetes, Deferribacteres, Euryarachaeota, Firmicutes, Plactomycetes, Proteobacteria, Spirochaetes, Synergistetes, Tenericutes and TG (Termite Gut) candidate phylum. The candidate TG phylum (1&3) and Fibrobacteres are the autochthonous gut symbionts of higher termites are found in the hindgut. Besides, many unclassified phylogenetic lineages within Actinobacteria, Firmicutes Proteobacteria and Spirochaetes were also found distributed in *Odontotermes* sp. Thus, the structural community data provides a basic information on the types of bacteria occurring in *Odontotermes* sp. Further, it also implicates the influence of diet and co-evolutional relationship among their respective ecological niches.

5. Genes and mechanisms underlying adaptation revealed using population genomic approaches - Oral

Why certain genomic regions resist gene flow in wood ant hybrids?

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Genomic comparisons between closely related species are identifying regions putatively underlying adaptation and speciation. However, recent studies have highlighted the inherent problems in these outlier scans: signals of differentiation may be artifacts of the methods used and can be caused by low recombination. Consequently, regions of high differentiation may not reflect loci underlying adaptation or speciation. Moreover, yet unexplored alternative is that multiple “speciation islands” arise because of epistasis, in other words because of genomic contingencies, where loci are not independent of each other even when they reside in different chromosomes. We investigate genomic basis of speciation using wood ant hybrids. Our previous studies have documented natural selection against gene flow in hybrid males as hybrid male eggs are laid, but they die during development. Loci underlying hybrid male breakdown are multiple and seem to be scattered around the genome. Currently, we are using population transcriptomics and genomics approaches to identify loci underlying hybrid male breakdown and reproductive isolation. However further work remains in determining if these loci show anys signs of adaptive evolution and whether they evolve independently from each other or under extensive epistasis.

Molecular mechanisms of caste determination in bumble bees

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Bumble bees (*Bombus*), like other eusocial insects, have queen and worker castes. Explaining the evolution and maintenance of castes represents a fundamental challenge in biology because the alternative phenotypes are distinct in their behaviour, morphology, and physiology, yet they both arise from the same genome. Bumble bees are a useful model for studying the evolution of caste determination in eusocial insects, because their caste systems vary considerably between species. Caste determination in one species, *Bombus terrestris*, passes through two critical phases in the larvae, a pheromone-dependent phase and a nutrition-dependent phase, whereas in *B. hypnorum* caste determination only has a single nutrition-dependent phase in the larvae. Furthermore, non-social, cuckoo bumble bees such as *B. vestalis* have secondarily lost their worker caste, and therefore their larvae have lost the ability to develop into more than one caste. In this study, our aim was to investigate the molecular mechanisms of caste determination in all three species. Specifically, we have used deep sequencing (RNA-Seq) of female larvae to isolate the genes that are 1) differentially expressed between castes in the two social species, 2) associated with the increased complexity of caste determination mechanisms in *B. terrestris* compared to *B. hypnorum*, and 3) associated with the loss of caste determination in *B. vestalis*. In this talk I aim to discuss the key results from these sequencing experiments. This study is the first to isolate genes associated with caste differentiation in a range of bumble bee species, each with a different system of caste determination.

Identification of multiple loci associated with social parasitism in honeybees

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Sex is the predominant mode of reproduction in animals. There are, however, examples where asexually reproducing populations may locally outcompete sexually reproducing ones. In one subspecies of the honeybee, A. mellifera, known as the Cape bee (A. m. capensis), worker bees reproduce asexually by thelytoky, where two nucleii produced by meiosis fuse to form single diploid eggs that develop into females. The Cape bee also exhibits a suite of phenotypes that facilitate social parasitism whereby workers lay such eggs in foreign colonies thus utilizing their resources. The genetic basis of thelytoky and its associated traits in the Cape bee is unknown. We compared genome variation in a sample of Cape bees with other African honeybee populations and perform a number of tests to identify signals of positive selection in the Cape bee lineage. We identify several regions of extreme genetic differentiation and signals of selection. These regions allow unambiguous genetic identification of Cape bees and likely underlie genetic basis of the traits that facilitate social parasitism. Further examination of these loci should give insights into the processes of reproduction, chemical signalling in both parasitic and non-parasitic populations and advance understanding of the process of normal and atypical meiosis.

Genome architecture of admixture and adaptation in the Africanized honeybee

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Genetic exchange by hybridization or admixture can make an important contribution to evolution. Introgression of favourable alleles additionally facilitate adaptation to new environments. Africanized bees are an admixed population of the honeybee (*Apis mellifera*) with characteristic highly aggressive defence behaviour that are the basis of a spectacular biological invasion. A small number of honeybees with mostly African ancestry were introduced to Brazil ~60 years ago, which began hybridizing with and replacing existing managed populations of European origin. These Africanized bees subsequently spread across much of the Americas. We characterised the genetic composition of 32 Africanized bees sampled throughout Brazil using whole genome sequence analysis. We show that these samples have 84% African ancestry with the remainder originating from western European populations. These proportions are consistent across geographical regions in Brazil suggesting that the population is relatively homogeneous. The distribution of ancestry tract lengths, when mapped to the genome, is consistent which indicates an average generation time of 1.5 years after the initial admixture event. Although Africanized bees have predominantly African ancestry, we identify one large 1-Mb segment on chromosome 11 where European haplotypes segregate at high frequency suggesting that they likely confer an adaptive advantage. The analysis highlights the processes by which populations of mixed genetic ancestry form and adapt to new environments.

Vitellogenin acts as a behavioural modulator in tapeworm-infected ants

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Parasite-induced alterations of host behaviours are often interpreted as being adaptive for the parasite in order to advance its own life cycle, and thus, suggests active manipulation of host behaviour by the parasite. Yet, alternative mechanisms such as host defence could also explain the observed alterations. One way to differentiate between these causes and to elucidate the mechanism behind host manipulation is to investigate the proximate basis of parasite-induced phenotypic changes. We analysed parasite-induced gene expression changes in the brain of tapeworm-infected ants, and used an RNA interference (RNAi) approach to clarify the function of three differently expressed Vitellogenin genes in infected and uninfected host ants. As a multifunctional protein, Vitellogenin not only regulates reproduction in social insects, but immunity, longevity and social organisation as well. Here, we will demonstrate how different Vitellogenin genes (Vit-1, Vit-3, Vit-6) affect ant behaviour and discuss the potential function of Vitellogenin genes for host manipulation or host defence. For example, Vitellogenin-6 was three-times higher expressed in infected workers, and RNAi-knockdown resulted in a reduction of brood care behaviour and an increase in other behaviours.

Patterns of speciation: differential gene expression underlying hybrid fitness

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Ants and other hymenoptera remain understudied in the context of speciation, yet their traits, namely a haplodiploid sex determination system, could prove to be an important tool for investigating the genetics of speciation. In this study we aimed to investigate an unusual pattern of hybridization in *Formica rufa* group ants from Southern Finland. In this system, previously studied by Kulmuni *et al.* (2014), there is a contrasting pattern of hybrid survival, with male larvae all dying, while hybrid females experience positive selection across their lifetime. To understand the molecular basis of hybrid male death and female survival we prepared barcoded transcriptomics for a total of 74 male and female larvae. Using this data we asked what gene expression patterns are associated with hybrid male death, and what gene expression patterns are associated with hybrid female survival. The intent of this appraoch was to reveal key genes putatively involved in hybrid male breakdown and female survival. The results of this study will contribute towards our understanding of speciation and hybridization in hymenoptera, as well as more broadly to our understanding of genome wide patterns of genomic incompatibilities.

Mollicutes bacteria: new mutualistic endosymbionts in fungus-growing ant guts?

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The attine fungus-growing ants are a monophyletic lineage that switched to an exclusive fungal diet ca. 50 MYA. We investigated the gut microbiota of a range of attine species and found that they are simple in most species. An unusual and little studied lineage of cell-wall-lacking Mollicutes bacteria appear to have important functions across the attine ants, as multiple strains are present across the phylogeny. To elucidate some of these putative functions we have genome sequenced two attine-specific Mollicutes strains: the leafcutter-ant-specific OTU EntAcro1 and the non-specific Apterostigma associated OTU EntAcro10, which is also present in moderate density in a number of other attine ants. Comparison with related Mollicutes strains of the genus Spiroplasma, known to be (often pathogenic) endosymbionts of other insects, showed that EntAcro1 and EntAcro10 have adapted to life in fungus-growing ant guts by utilizing metabolic pathways that are incomplete or absent in other Spiroplasma strains. The leafcutter specific OTU EntAcro1 showed the highest degree of co-adaptation to a life with fungus-growing ants, with several novel metabolic pathways that have functional roles in the acquisition/preservation of nitrogen and may complement the nutritional productivity of the hosts. After we recently documented a similar role for a Rhizobiales hind-gut symbiont, it increasingly appears that large-scale ant farming of an exclusive food-symbiont required the co-domestication of several, increasingly specific bacterial symbionts belonging to diverse bacterial orders.

Is there a link between miniature ant workers and the evolution of soldiers?

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Two kinds of ‘bigger helpers' exist in ants. In various species, workers vary greatly in size but growth rules are the same, hence small and large workers have different shapes due to allometry only. In other species (at least 15 formicoid genera), soldiers are a third caste with morphological traits (e.g. shape of head and mandibles) lacking in workers. In both cases, bigger heads reflect more powerful mandible muscles and specialized functions. Moreover, the bigger gasters and/or queen-like ovaries are associated with enhanced food storage and sharing in many species. Combining defence and trophic functions offsets the larger cost of manufacturing soldiers and majors.

The majority of ant species have miniature workers (Peeters & Ito 2015). In formicoids, miniature workers are often coupled with very big queens, thus a third caste corresponding to the intermediate morphospace can bring new benefits for the colony. However, soldiers do not exist in many species with tiny workers, and molecular phylogenies of the major subfamilies confirm a highly sporadic pattern of evolution (with the exception of *Pheidole*). In *Carebara*, some species nesting in buried branches have soldiers that can chew through rotten wood, unlike other species that dig underground chambers. The existence of queen and worker castes differing strongly in body size appears to be the developmental basis for evolving a soldier caste with a mosaic phenotype (e.g. phragmotic heads present in queens), but selective pressures are strongly influenced by cost-benefit ratios.

Discrimination of haploid and diploid males of bumblebees based on wing shape

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Being efficient pollinators of many flowering plants, bumblebees are an important group for temperate ecosystems services. Over the last decades they experience a strong decline in Europe because of different primary factors such as habitat fragmentation. These primary factors lead to genetic stresses that can reinforce the decline. This is particularly problematic in haplodiploid species, such as bumblebees, where diploids heterozygous at a single locus sex determination (sl-CSD) are females and haploids are males. Inbreeding can therefore lead to diploids homozygous at the sl-CSD which develop into functionally sterile males, which in turn leads to inbred strain having much lower fitness than outbred colonies. This is known as the “diploid male extinction vortex”. The monitoring of diploid males in wild population is therefore of primary importance for conservation management. Until now, diploid males cannot be morphologically discriminated from haploid males.

The present study aims to discriminate diploid males from haploid using wing shape and geometric morphometric methods.

Diploid males of Bombus terrestris were produced from sister/brother mating in different colonies, and ploidy was checked by flow-cytometry.

Based on wing shape, diploid males are accurately discriminated from haploid males using between-group PCA and LDA (hit ratio of 98% for males correctly attributed). Wing shapes of the other castes were also diagnostic (e.g. hit ratio of 100% for queens).

Conservation issues and potential applications are discussed. The morphometric identifiers can be turned into an automated or semi-automated identification tools suitable for field or museum studies. Morphology-based analysis will also facilitate future citizen-science bee monitoring schemes, e.g. involving smartphone or tablet based image analysis.

Statistical brain atlases illuminate the evolution of worker neuroanatomy

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Social brain theory, originally proposed for primates, hypothesizes that individuals living in larger groups evolved bigger brains than those in smaller groups (Dunbar and Schultz, 2007). This increased brain investment might enable individuals to adaptively process social information. Eusocial insects live in potentially huge colonies, but the relationship between worker brain size and colony size is unclear. For example, division of labor may result in reduced brains due to task specialization and thus fewer individual cognitive challenges (Gronenberg and Riveros, 2009).

To test this prediction, we analyze regional brain investment across ant species and worker subcastes. We have created archetypical templates of ant worker brains to statistical represent brain anatomy. Rather than pure averages resulting in low-resolution images, the templates are constructed through deformations of original samples. This results in (1) exceptionally clear images that represent ideal brains, and (2) maps of deformations that encompass variability across brains. These maps contain changes in size, shape and position of any region -or part of a region- and allow comparisons of samples that might vary in size or imaging conditions, collating them in the same normalized reference space. In addition, by adding expert annotations to the templates, we can create statistical atlases to automatically label new samples.

We use this tool to quantify 3D differences in brain investment among ant subcastes and species; our aim is to combine this information with phylogeny data to study brain evolution in association with social organization, ecology and behavioral specialization.

Evolution of Social Insect Polyphenism Facilitated by the Sex Differentiation Cascade

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The major transition to eusociality required the evolution of a switch to canalize development into either a reproductive or a helper, the nature of which is currently unknown. Following predictions from the ‘theory of facilitated variation’, we identify sex differentiation pathways as promising candidates because of their pre-adaptation to regulating development of complex phenotypes. We show that conserved core genes, including the juvenile hormone-sensitive master sex differentiation gene *doublesex* (*dsx*) and a *krüppel homolog 2* (*kr-h2*) with putative regulatory function, exhibit both sex and morph-specific expression across life stages in four morphs (queens, workers, winged males, wingless males) of the ant *Cardiocondyla obscurior*. We hypothesize that genes in the sex differentiation cascade evolved perception of alternative input signals for caste differentiation (i.e. environmental or genetic cues), and that their inherent switch-like and epistatic behavior facilitated signal transfer to downstream targets, thus allowing them to control differential development into morphological castes.

A method for profiling gene expression and sexing larvae in a cuckoo bumble bee, *Bombus vestalis*

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Explaining the evolution and maintenance of queen and worker castes in eusocial insects represents a fundamental challenge because these alternative phenotypes are distinct in their behaviour, morphology, and physiology, yet both arise from the same genome. Bumble bees (Bombus species) are a useful model for studying the evolution of caste determination, because their caste systems vary considerably between species. In *B. terrestris*, female larvae can develop into gynes or workers whereas those of *B. vestalis* (a cuckoo bumble bee) are unable to develop into more than one caste since this parasitic species has secondarily lost the worker caste. In this study, our aim was to investigate the molecular mechanisms of caste determination in female larvae of these two species using gene expression profiling by sequencing (RNA-Seq) and thereby to isolate the genes associated with the loss of caste determination in *B. vestalis*.

In *B. vestalis*, male and female eggs are both laid at the same time, making it impossible to identify female larvae in the colony. Another constraint was to obtain biological material in sufficient quality and quantity for the molecular experiment, since larvae of all developmental stages, including small early-instar larvae, were collected. Therefore, we performed a dual RNA/DNA extraction so as to extract from each larva enough i) DNA to determine the sex of the larva (using microsatellite genotyping) and ii) RNA to profile gene expression. I will present the method we used for the RNA/DNA extraction and key results from the genotyping and sequencing experiments.

Diploid male production results in queen replacement in the stingless bee *Scaptotrigona depilis*

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Like in most Hymenoptera, stingless bees (Meliponini) have a complementary sex determination (CSD) system. When a queen performs a "matched mating" with a male that shares a CSD allele with her, half of her diploid offspring are sterile, diploid, males rather than females. Previous research suggests that worker *Melipona* bees execute queens that have made a matched mating, thereby allowing a new queen to head the colony. Here we provide clear evidence that in the stingless bee *Scaptotrigona depilis*, the emergence of diploid males induces the queen death. The workers kill the queen in the colony within 10-20 days of the emergence of diploid male offspring from their pupae. Queens who have not made a matched mating are killed if introduced into a colony in which diploid males are emerging. This shows that the diploid males, not the queen who has made a matched mating, are what causes the workers to execute the queen. Analysis of the cuticular hydrocarbon profiles of haploid and diploid males shows twelve compounds with significant differences. This suggests that mother queens that have made a matched mating are detected based on chemical differences in the cuticular hydrocarbon profiles of diploid male offspring.

Stable eusociality via maternal manipulation when resistance is costless

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In many eusocial species, queens use pheromones to influence offspring to express worker phenotypes. Although evidence suggests that queen pheromones are honest signals of the queen's reproductive health, here I show that queen's honest signalling can result from ancestral maternal manipulation. I develop a mathematical model to study the coevolution of maternal manipulation, offspring resistance to manipulation and maternal resource allocation. I assume that (i) maternal manipulation causes offspring to be workers against offspring's interests; (ii) offspring can resist at no direct cost, as is thought to be the case with pheromonal manipulation; and (iii) the mother chooses how much resource to allocate to fertility and maternal care. In the coevolution of these traits, I find that maternal care decreases, thereby increasing the benefit that offspring obtain from help, which in the long run eliminates selection for resistance. Consequently, ancestral maternal manipulation yields stable eusociality despite costless resistance. Additionally, ancestral manipulation in the long run becomes honest signalling that induces offspring to help. These results indicate that both eusociality and its commonly associated queen honest signalling can be likely to originate from ancestral manipulation.

Vitellogenin-6 controls brood care behavior and behavioral maturation in ants

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In the complex societies of eusocial insects, workers specialize on certain tasks mainly depending on their age (age-polyethism). Whether an individual acts as forager or brood carer depends on the colony's needs and is modulated by individual variation in task thresholds, which are influenced by behavioral genes. How age and social environment interact to affect behavioral gene expression and consequently worker behavior is currently a hot topic in social insect behavior. Honeybees have been studied intensely in this respect including the identification of candidate genes such as *vitellogenin*, whereas ants have received less attention. To disentangle age and behavioral task, we manipulated the demography *Temnothorax longispinosus* ant colonies by removing either foragers, brood carers or workers of both castes. Subsequent comparisons of whole body transcriptomes detected more variation in gene expression in foragers. We will report further results of the gene expression analysis including functional clustering. We identified several candidate behavioral genes, including Vitellogenin-6, which was overexpressed in boodcarers. We consequently studied the phenotypic effects of vitellogenin-6 by RNAi knock-down. A 70% knock-down of vitellogenin-6 lead to a dramatic reduction in broodcare behavior, most likely achieved by a reduced sensitivity of workers towards brood related stimuli. Moreover, a long-term knock-down led to an accelerated behavioral maturation from brood care to adult nestmate care, which is a characteristic behavior of older workers. This indicates that the expression Vitellogenin-6 is a key player for the social organization within a colony.

The mechanistic, genetic and evolutionary basis of worker sterility in the social Hymenoptera

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Extreme reproductive skew towards particular females is a defining feature of the social Hymenoptera and workers are completely sterile in at least thirteen genera. The evolution of worker sterility is problematic because an individual that has decreased fertility has reduced direct fitness. In order to understand how worker sterility evolved it is essential to identify the mechanistic basis of worker sterility. We show that the developmental mechanisms that underlie worker sterility are 'reproductive control points' that reduce reproductive capacity in workers. We propose that environmental cues (nutritional and social) interact with particular signalling pathways in the worker and regulate worker fertility through reproductive control points both pre- and post-eclosion. There are eight gene signalling pathways that are likely to be involved in regulating worker fertility in honey bees: insulin/insulin-like growth factor 1 signalling (IIS); juvenile hormone; ecdysteroid; mechanistic target of rapamycin (mTOR); dopamine; mitogen-activated protein kinases (MAPK); epidermal growth factor receptor (Egfr) and wingless-related MMTV integration site (Wnt). We suggest that the common mechanism underlying all the reproductive control points is programmed cell death, an active process that causes the worker's reproductive organs to degenerate. These reproductive control points are likely to have been involved in the ancestral emergence of worker sterility from a solitary insect.

Superorganismality and caste differentiation as points of no return: how the major evolutionary transitions were lost in translation

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Four decades of sociobiology have left us with multiple superorganism concepts that are mutually inconsistent and uninformative on how superorganismality originates. These ambiguities can be traced to a broadened concept of eusociality that denied colonies with physically differentiated castes the special status that inspired August Weismann, William Morton Wheeler, Ronald A. Fisher and Julian S. Huxley to consider them as organism-analogs. The convenience definitions of superorganismality and eusociality that are commonly used preclude proper appreciation of which social insect lineages made irreversible evolutionary transitions to superorganismality and which did not. This has impeded straightforward connections between inclusive fitness theory and the major evolutionary transitions paradigm that emerged in the 1990s. Currently available comparative data are now helping to resolve these ambiguities in a way that is also applicable for explaining the origins of other post-eukaryotic transitions to higher levels of hierarchical complexity.

When to care about who to care for? Contrasting patterns of discrimination across taxa

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The ability of individuals to direct helping behaviour at recipients which are genetically more similar underlies kin selection. According to Hamilton's rule, all else being equal, costly forms of cooperation should be directed at closer relatives over more distant ones. While a body of work has discussed the mechanisms of kin discrimination in social insects, the mechanisms underpinning kin recognition and its relationship with helping behaviour in cooperatively breeding vertebrates remain poorly understood. For instance, where and when individuals should discriminate has been widely discussed in the context of costly nepotism in insects, but in vertebrates both data and theoretical conclusions are lacking.

We address this discrepancy by discussing the costs and benefits, and the underlying mechanisms of kin discrimination in vertebrates, and contrasting them to the understanding obtained from social insects. We focus on the cooperatively breeding banded mongoose, *Mungos mungo*, to highlight differences in the approaches traditionally used in studies of cooperation across taxa. We discuss recent findings demonstrating lack of kin discrimination in the banded mongoose. Our results point to marginal returns from discrimination in vertebrate systems where costs of nepotism and the high overall background relatedness may undermine potential benefits of discrimination among group members.

Neotenics: actors or spectators in termite eusocial evolution?

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In termites, the term "neotenics" designates individuals reaching reproductive maturity without going through the winged imaginal stage. This caste is obviously very ancient, since neotenics are nowadays widespread across most termite lineages. They have long been known to serve as emergency replacement reproductives in case of unexpected queen or king death, as supplementary reproductives allowing enhanced colony growth and expansion, or even as short-range dispersers for small-colony wood dwellers. Whereas the neotenics' importance in the reproductive biology of many species is undisputed, their role in the origin and evolution of termite sociality is much more controversial. Several scholars consider neoteny as a strategic novelty of termites, central to the evolution of their sociality because it provides immatures staying within their natal group with prospects of highly beneficial future reproduction. In contrast, I will review evidence for considering termite neotenics as an alternative flightless adult morph, comparable to brachypterous adults of other hemimetabolous insects. Adult wing dimorphism in termites would thus be much more ancient than the *Isoptera* themselves. Was this dimorphism a key factor promoting the evolution of termite eusociality? History is hard to reconstruct, but I will emphasize that major evolutionary transitions in the development of termite societies, which involve the specialization of immatures, may have occurred in complete independence from wing dimorphism among adults. Therefore, I suggest that it is the development of stable societies in termites that provided new and diverse opportunities for the expression of neoteny, rather than the other way round.

Aligning genetic interests and consequences for collective behaviors in ants

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In social groups, individuals may coordinate or self-organize to complete tasks vital to their fitness. While the mechanisms of such collective behaviors are becoming understood, we still know little about their evolution. A major driver of selection at the group-level is the degree of cooperation within groups, and thus, the alignment of fitness interests. To what extent do group-level traits, like collective behaviors, depend on cooperation and to what extent are they resilient to conflict? I investigate these questions in a slave-making ant that coordinates attacks on heterospecific ant colonies. I artificially reduced cooperation by elevating the proportion of egg-laying workers in colonies. I then compared raid performance between manipulated and control colonies. More cooperative colonies exhibit greater raid efficiency, however, colony size was a stronger predictor of efficiency. My results suggest that group size plays a more direct role in collective efficiency than cooperation.

Phylogenetic comparative analysis of factors driving transitions towards eusociality across the insects

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The evolutionary factors that drive the repeated transitions towards eusocial insect societies have been the source of heated debate, both historically and more recently (1, 2). Many theoretical models explore the contribution of preadaptations and genetical or environmental factors contributing to these transitions, yet we do not know the relative importance of these factors. To quantify how important various potential explanatory factors have been for (eu)sociality to emerge, we need to take a phylogenetic perspective on its evolution across all insects. We use modern phylogenetic comparative methods (3–6) to quantify relative importance of various potential preadaptations for eusociality. We show how these methods can help determine correlated evolution, order of events (what factors preceded and enabled eusociality evolution and what were later evolving refinements), and detect the phylogenetic signal of key preadaptations for cooperative interactions (7). Using a database of over 5000 insect species, we show that female monogamy and presociality, but not claustrality, are important preadaptations for eusociality. Current work is now analysing the relative contribution of more preadaptations, as well as genetic and environmental factors. This will help us provide a comprehensive account of why some insects became eusocial societies, while others did not.

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How did control of reproduction and sterile castes evolve in Apid bees?

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The defining characteristic of eusocial insects, including the honeybee (*Apis mellifera*), is the reproductive division of labour, where only one female caste is reproductively active.

In the honeybee the queen reproduces whilst worker bees are usually sterile and participate in brood rearing and other behaviours that ensure the success of the colony. For this division of labour to function, mechanisms have evolved to constrain reproduction in the worker caste ensuring this “altruistic” behaviour in worker bees. In worker honey bees, sterility or reproductive constraint is conditional; in the absence of queen mandibular pheromone (QMP) and brood pheromone, worker bees can activate their ovaries and lay unfertilised eggs. This process results in a complete remodelling of the small quiescent worker ovary, into a complex tissue containing all of the cell-types required to support oogenesis. I have previously investigated the molecular mechanisms that underpin reproductive constraint in the worker bee ovary and have identified several genes and signalling pathways that are key for this process.

We are now using other eusocial Apid species and solitary ancestors to understand how these essential mechanisms evolved to control reproduction in the worker caste of Apid bees.

Molecular adaptation in olfactory functions in the fire ant social chromosome

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Insects use pheromones as a major mode of communication. Social insects need a complex language of signals to coordinate cooperation between thousands of individuals. The evolution of such chemical communication required genes for the synthesis of pheromones and for their olfactory perception (receptors).

In the fire ant *Solenopsis invicta* chemical signals are involved in the determination of social organization. Monogyne or polygyne social structure is determined by the "social chromosome": a non-recombining region consisting of approximately 600 genes with two distinct haplotypes: *SB* and *Sb*. Monogyne queens are always *SB/SB* and polygyne queens are always *SB/Sb*. Workers discriminate monogyne and polygyne queens based on olfactory cues.

We searched for candidate genes in the social chromosome that could be responsible for this discrimination. We focused on olfactory receptors (ORs), because this gene family was dramatically expanded in ants. We annotated 461 putative ORs in the *S. invicta* genome. The OR gene tree shows many *S. invicta*-specific expansions, and multiple branches show evidence for positive selection. Notably, a cluster of 23 ORs resides in the social chromosome. Nine genes in this cluster are the result of recent duplications in the *S. invicta* lineage. We also identified significant differences in these genes between the *SB* and *Sb* haplotypes. Therefore, these receptors are prime candidates for involvement in queen discrimination. These results suggest that the evolution of polygyne social organization involved adaptations in genes responsible for olfaction and opens the way for functional studies of the molecular mechanism of this phenomenon.

A novel method for identifying imprinted genes in multiply-mated social Hymenoptera from RNA-seq data

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Over 100 genes in mammals show an unusual expression pattern where only the paternally or maternally inherited copy contributes to the phenotype, while its counterpart is silenced or shows reduced expression. The kinship theory of genomic imprinting states that this is a consequence of intra-genomic conflict between the two halves of the genome, a result of their differential relatedness to social partners. It predicts that imprinting may evolve wherever there are relatedness asymmetries and close interactions among kin. The social *Hymenoptera* therefore appear perfect candidates for genomic-imprinting, but so far no imprinted genes have been confirmed in any ant, social bee or wasp. We developed a method for systematically searching for imprinted genes in haplodiploid social insects whose queens mate with multiple males but that cannot be bred under laboratory conditions. We tested this protocol in the leaf-cutting ant *Acromyrmex echinatior*—providing the first genome-wide search for genomic imprinting in any ant species. We identified a number of genes as potentially imprinted and promising for further work, and we confirmed that Major Royal Jelly Protein 3 is not imprinted.

Unravelling queen pheromone sources in bumblebees

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Social Hymenoptera show a remarkable reproductive division of labour in which the queens reproduce and the workers remain largely sterile and raise the queen's offspring. However, since workers are also able to lay unfertilised eggs, a conflict of interests might arise within the colony which the queen smooths over by eliciting queen pheromones to serve as a signal of her fertility or as a mechanism to control her daughters' reproduction. In bumblebees, namely Bombus terrestris, the cuticular hydrocarbon pentacosane proved to be such a queen signal, but nonetheless a lot of inconsistency exists regarding bumblebee queen pheromone sources and their respective functions, of which the contentious role of mandibular glands as queen pheromone sources is an example. Here, we examined the effects of pure mandibular gland, Dufour's gland and cuticular extracts on worker reproduction and associated dominance behaviour in Bombus terrestris, and found that cuticular extracts reduced aggressive behaviour and surprisingly, mandibular extracts accounted for an increase in dominance behaviour and reproduction. The results of our research will provide a better understanding of possible queen pheromone sources in bumblebees and will further enlighten the evolution and conservation of reproductive division of labour in social insects.

Behaviour of males in primitively eusocial wasp socities: parasites or helpful brothers?

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Societies of eusocial Hymenoptera are often considered female-centric. The perception is diligent female workers maximise fitness by helping raise sisters, whilst males disperse for direct reproductive benefits. Models using this concept view males as detrimental on colony constitution, selfishly draining resources until dispersal. However, there is anecdotal evidence of helping-like behaviours observed in males: thermoregulation, defense, and broodcare. Are these pre-dispersal behaviours simply behavioural aberrations, of benefit to males only, or altruistic? We propose that males in these societies have a life history stage of contributing to colony well-being through these behaviours. Juvenile males could increase inclusive fitness by helping raise brood (indirect fitness), because mating opportunities are unavailable until sexual maturation (direct fitness). To test this, we quantified broodcare by males in the Neotropical primitively eusocial paper wasp *Polistes lanio*. When providing colonies with food supplements, males readily allocate resources amongst brood. This is negatively correlated with sexual maturation, observed in reproductive tract dissections. Through SNP genotyping, male relatedness to natal nest brood was explored, with queens in late-stage colonies maintaining control over workers in male production. We follow with investigations into what mechanisms may be involved in behavioural switching from a helping to reproductive male. This study hopes to illustrate possible novel aspects in the social evolution of Hymenoptera, and open up more discussions into potential adaptive roles of males in these primitively eusocial societies.

The importance of direct fitness for workers in a *Temnothorax* ant

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The evolution of insect societies and especially the division of reproductive labor has puzzled generations of scientists. Hamilton's theory of inclusive fitness shows that helpers can benefit from their apparent altruism by increasing the number of copies of their own genes by helping a relative to produce offspring. In addition to this indirect fitness component, helpers in societies of "primitively eusocial" insects and group-living vertebrates may also have the option to obtain direct fitness benefits. How important direct fitness is in advanced eusocial insects has rarely been quantified.

Workers of the monogynous ant *Temnothorax crassispinus* are capable of producing males from unfertilized eggs. Workers usually refrain from reproduction in queenright colonies due to worker policing and therefore do not gain direct fitness. However, after the death of the queen, workers form a hierarchy and the high-ranking workers gain direct fitness by producing sons. Preliminary studies revealed a considerable percentage of queenless colonies with reproductive workers under natural conditions. We currently determine the percentage of worker-produced males in the field and the reproductive success of these males.

Influence of queen and worker age composition on colony fitness

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Ant societies show complex division of labor between queens and workers, and often also between young and old workers. After the founding phase, colonies usually consist of workers of different age, which assign themselves to different tasks like foraging and nursing. Usually, younger individuals stay in the nest and care for the brood while older workers leave the nest and forage. Yet, there is some plasticity as, e.g. young workers may start foraging earlier when foragers are removed and vice versa. This flexibility has been suggested to be conducive to the fitness of the colony, but little is known about its concrete impact on colony performance. In addition, queens in polygynous colonies may also differ tremendously in age, which may have an impact on individual egg production. In this study, we investigate to what extend the age composition of queens and workers affects the reproductive success of colonies of the ant *Cardiocondyla obscurior*.

Smart wasps: Do the demands of a complex breeding strategy lead to increased investment in brain tissue in solitary wasps?

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The social brain hypothesis predicts that the evolution of more complex brains can be attributed to the cognitive demands of social life. Compared with other insects, hymenoptera tend to possess particularly complex brains. Hymenoptera are also by far the most social order within the insects, having developed eusociality multiple times independently.

Experiments to test the social brain hypothesis in this group have however met with mixed results. Other investigations have concluded that the cognitive demands of a parasitic lifestyle which preceded sociality have led to the development of brain complexity in this group. We present evidence from solitary digger wasps that the cognitive demands of particular breeding strategies may lead to brain development which could predispose these species to social life.

Specifically, these breeding strategies can be divided into those species which provision their larvae at the time of oviposition and then abandon them to their fate (mass provisioners) and those which perform extended parental care, returning to their hidden nests to reprovision their developing larvae periodically, requiring the capacity for spatio-temporal memory not experienced by mass provisioners. By using advanced Micro-CT scanning techniques we are investigating the development of different brain regions to search for a causal link between these cognitive demands and increased brain investment.

Can genes express communicational skills in the ant *Camponotus sericeus*?

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Many empirical and theoretical studies have focused on the frequency at which members of a group make use of information transfer among them, that is, social information use. Although social information use, in general, is considered highly beneficial, in certain species in which group living is the norm, members of groups do not always use social information but rather may engage exclusively in individual information acquisition. While there has been much experimental research on factors affecting the frequency at which a group makes use of information obtained from others, the evolutionary perspective has mainly been theoretically addressed. In the present study we conduct behavioural experiments coupled with functional genomic analyses to identify genes underlying the observed variation in social information use by the ant *Camponotus sericeus* (Forel). *C. sericeus* recruits via tandem running. The tandem pair are bound to each other by regular tactile bi-directional cues and also by a surface pheromone discharged by the leader. Interestingly, the bi-directional cues can range between tight communication to loose and in several cases to complete seperation between the pair. We predict that the difference between ants involved in communication and ants foraging alone is associated with changes in mRNA abundance in tissues of the head. Furthermore, the abundance of mRNA will be strongly correlated to the strength of communication between the leader and the follower. We strongly believe that these results will provide a starting point for elucidation of the evolution of the sophisticated communication seen in ant societies.

Dancing for their supper – Do honeybees adjust their recruitment dance in response to the protein content of pollen?

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Honeybees use the dance language to communicate the location of profitable food resources to nestmates. During nectar foraging, bees alter their dance to reflect the source's quality. As a result, the colony focuses on high-quality nectar sources. Here we ask whether foragers similarly adjust aspects of their dance when foraging for pollen according to the pollen's protein content. Pollen is essential for raising brood, and protein content varies substantially across plant species. We offered bees pollen, pollen substitutes or mixtures that differed in protein content and determined whether the duration of the return phase decreased and the number of dance circuits increased with increasing protein content. We further examined whether bees adjust return phase duration based on the protein content of naturally collected pollen. Lastly, we examined whether foragers are more likely to dance for pollen high in protein. Honeybees did not adjust the duration of the return phase or the number of dance circuits when mixtures contained more protein. Similarly, there was no relationship between protein content of natural pollen and return phase duration. Our results suggest that foragers cannot assess pollen's protein content. Bees were more likely to dance when collecting pure pollen, suggesting an important role of pollen-based cues in the regulation of pollen foraging. We will discuss the potential implications of the bees' apparent inability to select high quality pollen.

Aconitum and *Bombus* interactions: is floral rewards chemistry driving pollen-mixing behaviour in generalist bumblebees?

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Plants synthesise an array of chemicals to defend themselves against herbivorous insects. Some of these defense compounds also occur in nectar and pollen, which constitute resources for pollinators. Such floral chemicals may be beneficial or detrimental to bees: they may protect them against disease and pathogens but also cause toxicity. How these toxins affect pollinator behavior is not fully understood. Monkshoods (i.e. *Aconitum* species) are typical 'bee-pollinated' plants with large inflorescences and a specific floral morphology that makes their nectar accessible only for long-tongued visitors such as bumblebees. All the investigated species of *Aconitum* contain aconitine-like alkaloids, which are neurotoxic for insects. *Aconitum* pollen contains particularly large concentrations of alkaloids compared to the leaves or the nectar. These chemicals may protect the flower from herbivores or deter generalist pollen-collecting bumblebees. A recent study suggested that pollen mixing in generalist bees might be considered as a possible strategy to exploit flowers with unfavorable pollen. To elucidate to what degree generalist bumblebees mix pollen in *Aconitum-Bombus* interaction system, pollen loads from two generalist species (*Bombus hortorum* and *B. wurflenii*) were collected in several sites with different *Aconitum* species for chemical analyses. In addition, analyses were conducted on pure pollen and nectar of the *Aconitum* species, pollen loads from one *Aconitum*-specialist bumblebee (*B. gerstaeckeri*) and bumblebee tissues. Comparison between the different data allowed us to investigate potential alkaloid sequestration in bumblebees as well as potential dilution mechanisms of *Aconitum* pollen that could lead to alkaloid concentrations too low to affect generalist survival.

Nutrition mediates the expression of cultivar-farmer conflict in a fungus-growing ant

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The ant farmers (Tribe: Attini) provide fascinating parallels with human farmers, having domesticated their fungal crops for improved nutrition over millions of years. While evolutionarily derived leafcutter ants achieved industrial-scale farming, extant species from phylogenetically basal attine genera continue to farm loosely domesticated fungal cultivars capable of pursuing independent reproductive interests. We developed geometric framework feeding experiments with the basal attine *Mycocepurus smithii* to test whether reproductive allocation conflicts between farmers and cultivars constrain crop yield, possibly explaining why their mutualism has remained limited in scale and productivity. Our results show that carbohydrate-rich substrates maximize growth of both edible hyphae and inedible mushrooms, but that modest protein provisioning can suppress mushroom formation. Worker foraging was consistent with optimizing rather than maximizing cultivar performance: Ant farmers could neither increase carbohydrate provisioning without cultivars allocating the excess towards mushroom production, nor increase protein provisioning without compromising somatic cultivar growth. Our results confirm that basal attine farming has been very successful over evolutionary time, but that unresolved host-symbiont conflict may have precluded these wild-type symbioses from rising to ecological dominance. That status was achieved by the evolutionary derived leafcutter ants following full domestication of a co-evolving cultivar ca. 30 MY after the first attine ants committed to farming.

Impact of pollen ressources drift on common bumblebees in NW species

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Bumblebees are major pollinators in several wild ecosystems and agricultural landscapes. Their conservation is a major concern as recent studies have pointed a decline of the populations of many species. This decline could be caused by a shift in host-plant quantity and quality through last decades. We tested this hypothesis by considering five common species of bumblebee in NW Europe (*B. hortorum, B. lapidarius, B. pascuorum, B. pratorum* and *B. terrestris*) for which we had a description of their pollen diet through two time periods. For each species we estimated the shift in their pollen diet as well as the change in the suitable area of their pollen resources to assess the relation between the two parameters. Our hypothesis was that common bumblebee were able to shift on more abundant resources. Concurrently we evaluated whether the chemical composition of pollen resources changed over time and experimentally tested the impact of new major pollen species on the development of colonies.

Results show that only *B. lapidarius* significantly included more pollen from spreading resources. Chemical analyses reveal that amino acids pollen content were stable between the two periods but depend on plant diversity. Rearing results have pointed the better development of colony fed on *Trifolium repens* pollen, a major resource in the actual diet of bumblebees.

Overall the present study shows that the response to resource drift varies with species, even among common closely related pollinators, and that species diverse plant community ensures stable chemical quality of pollen diet for generalist species.

Enzyme capacities to decompose plant biomass in microbial symbionts associated with fungus-growing termites

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Fungus-growing termites in association with the mutualistic fungus *Termitomyces* and gut bacteria are the main plant decomposers of the Old World. The termites predigest plant material along with fungal nodules before inoculating their faeces as fresh fungus comb, within which *Termitomyces* decomposes the plant material. The mature fungus comb passes a second gut passage, where gut microbes finalize the decomposition, after which the plant substrate is essentially completely degraded. Using different approaches we aim to better understand co-evolutionary adaptations in *Termitomyces* and gut microbes with respect to plant decomposition capacities. Analyses of carbohydrate-active enzyme expression using RNAseq on nodules, fresh comb, and old comb from *Macrotermes natalensis* showed that cellulases are highly expressed in fresh comb, less so in old comb, and least in nodules. Since primary faeces is inoculated on the fresh comb, high cellulase expression is expected for *Termitomyces* to gain nutrients for growth. AZCL and chromogenic substrate enzyme activity assays on nodules, fresh and old comb, and termite guts showed that activities were, however, generally higher in nodules than in combs, while guts were similar to nodules. This suggests that enzymes may remain active during the first gut passage to be transported from the old to the fresh comb, which could facilitate faster decomposition of the recently incorporated plant material. Further experiments and analyses are underway to explore the division of labour for plant decomposition in this tripartite symbiosis, including across additional termite species and their symbionts.

How to taste pollen quality: bumblebees use chemotactile cues to assess pollen nutritional composition

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Numerous studies have investigated visitation patterns and floral preferences of bees, whereas the proximate mechanisms underlying bee foraging choices are still poorly understood. In fact, there is ongoing debate on whether and how bees assess the nutritional quality of pollen, or whether information on pollen quality is used to adjust individual foraging patterns.

To better understand whether and how a primitively eusocial bee species, Bombus terrestris, assesses pollen quality, we used chemotactile conditioning of the proboscis extension reflex (PER) and feeding choice assays to test whether bumblebees are able to differentiate between pollen of different protein/amino acid concentrations and whether they choose pollen of higher protein concentrations. We further applied chemotactile electro-antennogram (EAG) recordings and chemotactile PER conditioning to examine whether bumblebees can discriminate between different essential amino acids.

Our results show that individual Bombus terrestris workers use their sense of taste to discriminate pollen differing in concentration and thus nutrient content and use this ability to always prefer pollen of higher quality. Moreover, they continuously re-assess quality, as artificially changing pollen quality resulted in rapid equivalent changes in foraging behavior, even without feedback from larvae. As bees are also able to differentiate between different amino acids, they may use specific amino acids as cues for assessing pollen nutritional quality, besides or in addition to other nutritional compounds (e.g. steroids, fatty acids). We therefore suggest that individual bumblebee foragers continuously re-assess pollen quality based on free amino acids to provide their colonies with high quality food.

The behavioral mechanism of collective food intake regulation in *Camponotus sanctus* ants

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Ant colonies have been shown to tightly regulate their collective food intake - an impressive control considering that only foragers convey food to satisfy the needs of the entire colony. Despite accumulating experimental evidence for this tight regulation, theoretical suggestions, and indications from other social insects, we still know very little about the behavioral mechanism underlying this decentralized control. Here we present data from observations, as well as experimental manipulations, on colonies of individually barcoded *Camponotus sanctus* ants who feed on fluorescent food. With the food flow made visible upon the trophallactic dynamics, we directly examine the behavior of the foragers with respect to the satiety state of the colony. Perturbing the system and studying the foragers' response, we further infer about the individual behavioral rules which govern the collective intake regulation.

Resource redistribution in polydomous ant nest networks

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Social insect colonies are often viewed as central place foragers. Certain ant species, however, spread their colonies out across multiple socially connected, but spatially separated, nests. One possible function of the social connections between nests of the same colony is to allow resource redistribution, correcting nutritional imbalances between the nests. We studied a polydomous population of the wood ant *Formica lugubris* over 4 years, and found that nests differed hugely in their foraging activity, with some carrying out no collection of carbohydrate from foraging at all. The ants in these nests acquired carbohydrate through trophallaxis with ants in neighbouring connected nests. We found that trails connecting nests that differed greatly in their foraging income were stronger than trails connecting more nutritionally balanced pairs of nests. Using network analysis, we investigated the importance of a nest's position in the resource-flow network on life-history characteristics of the nests. Our results show that nests located in network positions that make them important for resource flow are more likely to survive, grow and bud off new nests than are other nests in the same network. This dynamic process of nest abandonment and establishment results in a self-organised continual adaptation of the nest network to the nutritional environment. Our results show that to understand the 8. Nutritional ecology of polydomous ant colonies, we must investigate not only what occurs within an ant nest, but also the dynamics of resource flow between nests that are acting in cooperation.

Nutritional status and division of labor in a clonal ant species

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In social insects, colonies are characterized by a division of labor, with one or a few individuals specializing in reproduction, and the majority of the colony members - the workers - handling all other tasks. However, the proximate factors underlying task allocation and specialization in social insects are not yet fully understood. Clonal ants are ideal models to investigate the proximate mechanisms underlying division of labor, while avoiding other confounding factors, e.g. genotype. In the clonal ant Platythyrea punctata all individuals are morphologically and genetically identical. Nevertheless, its colonies are characterized by a well-ordered reproductive division of labor based on rank orders established by young workers through fighting. As a consequence, each colony contains only one, rarely several, reproductive workers, while the majority of individuals has inactive ovaries. What initially determines rank differences among clone-mates of similar age remains unknown.

In this study, we investigated if there is a link between the worker nutritional status and division of labor. By setting up standardized experimental colonies and using food manipulation, we studied how stored lipid contents influence reproduction and foraging decisions. We found that workers from callows to foragers differ in fat content. By controlling worker ages, we showed that lipid levels were a good predictor of the onset of foraging and egg laying capacities: starved workers were more aggressive than better-fed worker but almost never became reproductive individuals. Finally, we demonstrated that increase in fat content happen in foragers that revert to nursing.

Tracing the flow of nutrients through the complex symbiotic network of the leafcutter ant *Atta colombica* using stable isotopes

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The ecological success of social insects is governed by the distribution of nutrients within their societies. Fungus-farming ants (Tribe: Attini) channel nutrients through an additional symbiotic partner, using cultivated fungus to convert recalcitrant substrates into usable nutrients. Domesticated cultivars generated by evolutionarily derived leafcutting ants concentrate nutrients in specialized organs called gongylidia. Ant foraging behavior typically aims to balance harvest of protein and carbohydrates, but little is known about the internal regulation and distribution of these nutrients in the ant-fungus symbiosis. We modified geometric framework protein:carbohydrate diets to include isotopic tracers (^{15}N and ^{13}C) and track nutrients within colonies of the leafcutting ant, *Atta colombica*. This enabled us to: 1) Measure the turnover time during which nutrients harvested by ants are assimilated in gongylidia, 2) Determine if harvested nutrients bypass the fungal cultivar en route to assimilation by workers and/or brood, and 3) Reveal if workers regulate nutrition by differentially disposing protein or carbohydrates from harvested substrate in the trash pile. Preliminary results allow us to infer both the rate of nutritionally transfer from harvested substrate to brood that need a high supply of nutrients to accumulate new body tissue, and to workers that need energy for foraging, defense and garden maintenance. These results indicate that enrichment of the system occurs rapidly, constaly producing new fungus material to support the large number of individuals in colonies of leafcutter ants. More generally, this research maps the nutritionally transactions that enable an industrial-scale farming system with an enormous ecological footprint.

Tracking food economics in bumblebee micro-colonies (*Bombus terrestris*) using lanthanides

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Energy flow and costs are not yet fully understood in social bees. Effectivity of sugar and pollen utilization and complexity of the nutritional flow in a bee colony indicate a need for a suitable experimental approach of food distribution monitoring throughout a colony. We introduce a new method of sugar and pollen tracking that utilises an inert lanthanide complex (LnDOTA or LnDTPA).

We used artificially bred *Bombus terrestris* for our experiments. Micro-colonies consisted of three workers had unlimited acces to sugar (saccharose, fructose and water solution) and pollen. Sugar solution was labeled with GdDOTA (for control colonies equivalent amount of water). The sugar solution with lanthanide was transported by workers throughout the colony and to the larvae. The delayed defecation of bee larvae enabled the collection of all faeces from a cocoon. The amount of sugar digested by larvae during development correspond to the amount of the lanthanide in the faeces, which was quantified using ICP-OES techniques.

We proved information that this method was optimal for monitoring food distribution and that it had no negative effect on the development of the colony. The results show that bumblebee males ingest 29 mg of sugar per 10 mg of its body weight for their development. It amounts 128 mg of sugar per individual in average.

We highlight the possibility of this method to be extended for tracking flow of different components of food within a colony using up to 15 different lanthanide markers without the necessity of killing individuals.

Are commercially produced bumblebees necessary in strawberry crops?

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Over a million bumblebee colonies are commercially produced and exported globally in order to provide crop pollination services that claim to increase yield and enhance the quality of agricultural crops. Every year, in the UK, approximately 15,000 *Bombus terrestris* colonies are imported for use on soft fruit farms. Strawberry is an economically important soft fruit crop in the UK, with production estimated to be worth £244 million in 2014, and use of commercial bumblebees on strawberry farms is common practice. However, despite this widespread use, there is little quantitative evidence that they are providing a benefit to farmers. Given the negative consequences associated with importation of commercial colonies (e.g. disease spread, interbreeding with wild populations), it is vital that the benefits of commercial bumblebees are quantified, so reasoned management decisions can be made that provide maximum benefit to both farmers and wild bees. In this study commercial bumblebee nests were placed into open ended polytunnels on a strawberry farm in the south east of England. The entrance to the nests were opened and closed on a weekly cycle. The diversity of wild bumblebees was surveyed, and fruit was harvested and later quality assessed. The quality and parasite levels of the colonies was also measured. The results will help clarify whether commercial bumblebees increase the yield and quality of strawberry crops, or whether adequate pollination is carried out by wild bumblebees and other pollinators. This could have implications for the conservation of wild bumblebees and for soft fruit farm management practices.

Exploration vs exploitation: life-long tracking of bumblebee foraging routes

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Insect pollinators such as bumblebees play a vital ecosystem role, so it is important to understand their foraging movements on a landscape scale. Recently, technological advances have made life-long animal tracking a possibility, with the potential to revolutionise our understanding of animal movements. We used harmonic radar technology to track every flight of bumblebee foragers (Bombus terrestris audax) through their entire lives, allowing us to tell the ‘life stories' of individual bees in unprecedented detail. Our data comprise 244 complete flights, encompassing more than 15000 minutes of flight and covering a distance in excess of 180 km. Analysis of this dataset reveals extreme levels of variation between individual bees in their foraging effort, how they explored their environment and the way they balanced exploration with exploiting learned forage patches. Exploitation of learned resources takes place during efficient, straight trips and is seldom combined with exploration of other areas. Exploration of the landscape typically occurs in the first few flights, but further exploration can take place throughout the bee's foraging career. One bee followed a straight route to a forage source exclusively for six days, finally abandoning it for a closer location. This second location was apparently remembered from her initial exploration flight, nine days earlier. Another bee continued to explore widely throughout its life, while two other bees showed frequent switches between exploration and exploitation. Our data show how bumblebees balance exploration of the environment with exploitation of resources and reveal the extent of variation between individuals.

Mapping hotspots of environmental stress to wild bees across Great Britain

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Land-use change (LUC) to agriculture is considered a primary driver of wild bee declines. In Great Britain (GB), >77% of land surface is agricultural with 1/3 being cropland. Cropland is associated with floral diversity loss, foraging and nesting habitat fragmentation, and presents a pesticide exposure landscape. These factors may interact to threaten bee populations, yet ironically, human population growth and subsequent increased demands for pollination services likely places bees under increasing stress.

Effective planning for bee conservation on emerging cropland requires an understanding of the spatial distribution of cropland and associated hazards, and to anticipate future change. To address this we developed a novel LUC model, with 222,257 x 1km^2 pixels covering 98% of GB, which predicts 1/5 of land currently covered in woodland and grassland, which can support bee populations, is likely to become cropland by 2040. We further linked the model to a comprehensive cropping and pesticide dataset to produce heat-maps elucidating ‘hotspots of stress' to wild bees. Furthermore, to ground-truth our model we are carrying out extensive field-sampling of wild bees in predicted areas of differential risk.

Our study is novel as we combine modelling and field-based approaches to link local, site-level processes to national patterns of insect pollinator decline. Our results can be used at multiple scales, from local, even pixel, level to guide land management and conservation actions, through to overarching national policy. The results enlighten the discussion around the future of wild bee conservation and food security.

Sociality, body size and diet breath as predictors of population genetic structure in bees

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Understanding population structure is key to developing predictions about species susceptibility to environmental changes, such habitat loss and climate change. Bees have recently become the focus of conservation concern due to increasing evidence of population declines worldwide, drawing attention to the ecological and economic consequences of pollinator loss. In this study, we used a comparative phylogenetic approach to assess the extent to which species-specific biological traits affect patterns of population genetic structure. We reviewed the current literature on population genetics of bees (n = 63 studies) and fitted phylogenetic generalized linear models to assess the effect of sociality, body size and diet breath on their genetic differentiation. The effect of sociality was not overall significant, though mean genetic differentiation in solitary species was greater than in social species. Body size had a subtle, though significant, negative effect on genetic differentiation. We did not find any consistent effect of diet breath. Our results highlight the importance of body size in shaping population genetic differentiation and suggest that undertaking standardized population genetic analyses will help identify the most susceptible species to habitat and climatic changes.

Invasive Synergy in an ant-plant mutualism

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South Africa is home to one of the world's six floral kingdoms, with over 6200 endemic plant species; yet this unique region is threatened by a number of invasive plant and animal species, including the notorious Argentine ant (*Linepithema humile*). This ant species threatens not only the native ants' community structure, but also the ecosystem services they provide. This study explores the seed dispersal ability of invasive and native ant communities. Through the use of cafeteria experiments and nest excavations, ant dispersal preferences were determined for both native fynbos and invasive *Acacia* plant species.

Three ant communities were identified in this study. The community invaded by *Linepithema humile* were able to disperse a range of smaller seeded plant species, with the highest rate of removal recorded for three invasive *Acacia* plant species. In contrast, the native community dominated by *Anoplolepis custodiens* showed a preference for larger native seeded species, with the lowest removal rates recorded for *Acacia* plant species. Whereas the native community dominated by *Pheidole capensis* showed no clear preference for either native or invasive plant species.

In conclusion the invasion of *Linepithema humile* is likely to facilitate the invasion of *Acacia* plants, whilst at the same time limiting the dispersal of native fynbos species, in particular larger seeded genera, such as *Leucospermum*. This invasive synergy highlights that growing threat that ant invasions pose and the urgent need for conservationists to consider a wider number of influential factors when determining the extent of their impacts.

The occurrence of the shining guest ant in red wood ant nests

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The shining guest ant *Formicoxenus nitidulus* is an obligate social parasite, and thus, its survival is strictly tied to its hosts. Although it has several different host species (mound-building *Formica*), and a wide Transpalaearctic distribution, it has globally been classified as vulnerable according to the IUCN Red List. To effectively conserve this species, it is important to understand its habitat requirements, especially as not all potential host nests are occupied by the guest ant. We investigated how host species, nest mound size, inter-nest distance and nest density relate to the occurrence of *F. nitidulus*. In total, we surveyed 166 red wood ant nests (*Formica rufa* group). Overall, *Formicoxenus nitidulus* was found in ca. 60 % of the nests. Only *F. polyctena* and *F. rufa* nests were included in the analysis due to the small number of other nests. *F. nitidulus* was more likely to be found with the polydomous *F. polyctena* than the monodomous *F. rufa*. In accordance with previous studies, we found *F. nitidulus* to prefer larger host nests. Furthermore, while inter-nest distance was not important, *F. nitidulus* preferred high nest density, and especially the well-connected nests in polydomous colonies. Thus, the best habitat for the shining guest ant is a dense population of mounds with a high proportion of large mounds. However, while large nest mounds are most optimal for the guest ant, small and medium sized nest mounds ensure the continuum of large nest mounds in a population also in a longer time span.

Firsts results on the impact of the invasive yellow-legged hornet *Vespa velutina* on natural communities and ecosystems

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The yellow-legged hornet *Vespa velutina* Lepeletier 1836 is an Asiatic hornet species introduced in France in 2004 that is colonizing many European countries. In Italy, since its arrival in 2012 and 2013 with the detection of first adults and nests respectively, the species is quickly spreading throughout the northwest part of the country. *Vespa velutina* is considered an invasive alien species because of its impact on natural ecosystems, apiculture, and human well-being. In fact, *V. velutina* actively preys on honeybees, wild bees, and other native insects and thus could disrupt natural communities and ecosystem equilibrium. For these reasons, monitor and control activities recently started in Italy, thanks also to the contribution of an European Life Project (LIFE14 NAT/IT/001128 STOPVESPA) "Spatial containment of *Vespa velutina* in Italy and establishment of an Early Warning and Rapid Response System". Quantifying the impact of an invasive alien species is a fundamental process for the establishment of management plans at a national or international level, because data on biodiversity and economic losses are requested by decision-making bodies. Consequently, one of the aims of the STOPVESPA project is the evaluation of the impact of *V. velutina* on natural pollinator communities and the ecosystem services they provide. We therefore stablished a monitoring network of areas with and without the presence of *V. velutina*, where the insect community is sampled at regular intervals. In this work we present the most relevant results obtained in the first year of activity.

Breeding for pollinator friendly crops: the case of the field bean (*Vicia faba* L.)

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In agricultural landscapes, mass flowering crops such as the field bean and oilseed rape provide a large but temporary foraging resource to pollinating species. These flowering crops have the potential to improve pollinator populations, if flowering coincides with key stages in the pollinator's lifecycle. However, breeding programs for flowering crops do not directly consider how floral traits affect the attractiveness and overall resource provided by the plant to pollinators. Therefore, crops may be suboptimal in their value to pollinators.

This investigation aimed to identify floral traits that had the most potential to improve the attractiveness and overall reward of the field bean to its bee pollinators. From a large panel of genetically distinct lines, we investigated the natural range of heritable variation in floral traits. From these lines it was demonstrated that there is great potential to breed for flowers with greater reward value. This could be achieved not only by improving the nectar content of flowers, but also by improving the accessibility of the reward to bees by reducing the force required to open flowers. Reducing the corolla-tube length may also allow shorter-tongued bees to access the nectar of flowers. Using bee behavioral experiments, we have investigated how the tradeoff between sugar concentration and solution viscosity affects the preference of Bombus terrestris.

As the yield of the field bean has been shown to be improved by bee visitation in numerous studies, we believe this approach of breeding more pollinator-friendly flowers should benefit both farmers and pollinators.

Effects of soil disturbance on ant-seed interactions in roadside woodland remnants in southern NSW, Australia

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Seed dispersal by ants is a common ant-plant mutualistic relationship worldwide. Attracted to a food reward, called elaiosome, ants move seeds into their nests to consume the elaiosome, and then discard the seed into the surrounding area. Habitat disturbance is known to alter the success of this mutualism by influencing the composition and behaviour of ant communities, however little is known of the effects of soil disturbance. In much of south-eastern Australia, past land-use has resulted in extensive clearing of previous woodlands for agricultural purposes, where remaining patches of remnant vegetation exist mainly in roadside environments. Many roadsides are now of high conservation status, yet regularly disturbed by (soil) disturbances from road management activities (i.e. road grading operations).

Field studies were conducted in a typical fragmented landscape which contained a large network of minor rural roads and associated remnant vegetation in southern NSW, Australia. 24 road segments were selected that each contained an undisturbed and a disturbed (by road maintenance) zone. Seeds of Acacia pycnantha were offered to ants at multiple ‘cafeteria' bait stations in both zones at each site to conduct observations on ant-seed interactions. Despite a large number of ant species were observed interacting with seeds, two ant species, Rhytidoponera metallica and Iridomyrmex purpureus facilitated most seed removals. Mean seed dispersal distance was greater in the disturbed zone (mean 12m, maximum 120m), largely due to the longer foraging ranges of meat ants (I. purpureus), which appeared to thrive in habitat conditions as a result of soil disturbances from roadworks.

The role of ants as bioindicators in the Biochar-soilfauna interplay

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Biochar is charcoal produced by pyrolysis and used as soil amendment in agriculture. Its use is now increasing because it is considered a useful tool in crop productivity enhancement and together it has been proposed as a climate change mitigation strategy. However, biochar induces changes in soil properties that are known to have a direct impact on soil ecosystem with consequences for soil biota community that, in turn, can influence biochar aging in soil. Despite several studies investigated the interplay between biochar and soil microbiology, there is a clear lack of information on groups that live in the most superficial ground layers. Our study is one of the first field attempt to investigate the interactions between biochar soil amendments and aboveground soil macro-meso fauna. In summer 2013, we set-up a randomized-block experiment on a processing tomato crop in northern Italy. We used three different biochar types, periodically monitoring soil parameters and fauna abundances along the crop growing cycle. Results show that this amendment does not have short-term ecological interferences. Nevertheless, ants exhibited variations in abundances and distribution connected to properties of amended soils such as temperature, pH and humidity, proving that they can be effectively used as a target group in the study of interactions between biochar and soil biota.

Contributing to the knowledge and management of the invasive hornet *Vespa velutina*: sexual communication and competition with native hornets

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The hornet Vespa velutina (hereafter Vv) was spotted for the first time in 2004 in France. Due to its huge invasion potential, habitat flexibility and specialization in honeybee predation, Vv represents a high-concern species both ecologically and economically. Despite such a great interest, many knowledge gaps still exist. Here we show our preliminary results related to: i) investigation on the possible existence of sexual pheromonal communication and ii) evaluation of the potential competition between Vv and the native hornets V. crabro (hereafter Vc), which shares a very similar ecological niche. In the light of the development of specific attractive odorants for monitoring and mass trapping, we investigated, thanks to behavioural, chemical and electrophysiological assays, the possibility that Vv reproductive females emit pheromones attracting males, as it seems to occur in other Vespinae species. While preliminary results suggested a male preference for reproductive females, we did not find evidence of a male response to potential female attractive odorants. In order to assess potential competition, we investigated two life history traits that might pose the two species in competition: a) the ability to find food sources and the flexibility in exploiting them, and b) the immune-competence response that might give advantage in terms of pathogen resistance. Our results show potential competition over resources and differences in immune-competence and set the stage for future investigations on these poorly investigated topics.

The effect of polyandry on melanin-based colouration of the red wood ant *Formica rufa*

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Animal colouration possess important adaptive meaning in camouflage, signalling, mimicry, physical protection and temperature regulation. In insects, dark cuticular colour is frequently dependent on melanins - pigments, which are involved into numerous physiological processes, such as physical defence, thermoregulation, desiccation resistance and immunity. Depending on species and the functional role, production of melanin-based colouration can be environmentally dependent or genetically determined. Offspring of a singly or multiply mated single queen in ants could reflect genetically determined paternal effect in colouration. We studied colouration of workers of the red wood ant *Formica rufa* that is mainly monogynous with variable mating frequency in its Finnish populations. The workers exhibit vast variety of modular melanin-based colour variations, including distinctly visible colour morphs on head and pronotum. Head and thorax deposit melanins in opposite ways. Mean darkness and area of melanisation show increase with an increase in head size, while with the size increment darkness of thorax decreases. Melanin-based colouration in workers of *F. rufa* was not associated with the level of polyandry and might be produced by the environmental factors. Colour patterns of red wood ants may be useful as biomarkers of environmental stress in bioassays.

Distribution of wood ant mounds in the Białowieża Forest

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Wood ants are common in coniferous and deciduous forests of the Palaearctic ecozone. Because of their high numbers they are one of the most important groups of insects in most environments. The Białowieża Forest (62 219 ha) is the best preserved forest ecosystem within the temperate zone in Europe, and widely regarded as a model forest, related to observations and research into pristine deciduous and mixed forests. Studies concerning the distribution of wood ants on a relatively large scale have been conducted in the Belarusian part of the Białowieża Forest. There is a lack of knowledge about the distribution of wood ants in the Polish part of the Białowieża Forest. Thus our aims are: a) to carry out an inventory of wood ant mounds and determine the species composition of wood ants occurring in the Białowieża Forest District; b) to determine the preferences of wood ants to forest parameters, such as tree stand composition, habitat type and exposure. The inventory of all active and abandoned wood ant mounds in the Białowieża Forest District began in spring 2016. The following ant mound parameters are determined: diameter at the base, mound height and exposure of the longest slope. From all active ant nests 10 workers are taken to identify the species. The project started in March 2016 and will last until 2018. The results of this study can be useful when planning measures for protecting wood ant colonies in the temperate forests of Europe.

Controlling the invasive garden ant (*Lasius neglectus*)

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Invasive alien species are responsible for extensive damage to ecological systems as well as significant economic costs. The invasive garden ant (*Lasius neglectus*) has emerged as an invasive pest in the last three decades and is causing widespread problems in Europe. The distribution of *Lasius neglectus* is increasing exponentially and its biological characteristics make it difficult to control. Previous field based attempts to control the ant have had only limited success. We performed a laboratory-based palatability and mortality experiment aiming to identify a pesticide suitable for the control or eradication of the invasive garden ant over large areas. Our results show that commercially available pesticides vary hugely in their efficacy as regards the invasive garden ant. Gel application is more effective than granular, but granular is easier to administer. The best strategy for controlling this ant is discussed.

Community of eusocial bees living in cacao plantations of the state of Bahia, Brazil.

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The importance of bees in the environment can be measured by their participation in the plant pollination as well as their use for biomonitoring due to their sensitivity to environmental changes. A current concern is about the conservation of native stingless bees (Meliponini). The aim of this study was to identify the species of eusocial bees living in cocoa fields in southern Bahia, Brazil, despite their inability to pollinate that plant. Three cocoa experimental plantations of 1 ha each, divided into 150 plots, shaded by exotic trees of the legume genus Erythrina and located at Ilheus were sampled between 11/2012 to 12/2013. Active search was performed for 30 minutes per plot. Furthermore four Malaise traps were installed in the field for 10 days. The active search allowed collecting 76 individuals, while traps recorded 29 occurrences. 15 species of eusocial bees in 11 genera were recorded highlighting Plebeia and Trigona and the exotic Apis mellifera scutellata. These bees rarely forage for pollen on the cocoa tree itself, but may use foliage and at least eight species of plants (shading trees and bromeliads). Bees have also been spotted exploring other resources, such as foragers of Trigona hyalinata collecting clay. Despite being a monoculture in which few native plants are maintained, this kind of cocoa plantation supports a significant diversity of eusocial bees. The closeness of the Atlantic Forest makes it a favorable environment for the maintenance of eusocial bees, making this agroforestry system an important ecological corridor.

Detection and destruction of fatal infections in ant colonies

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Evolutionary theory predicts that animals able to identify sick conspecifics will have a higher fitness if they can use that information to reduce their own risk of infection. As close contact between conspecifics is a key transmission route for infectious diseases, social insect colonies should be under strong selection to identify sick nest mates and respond in a way that prevents single infections turning into a colony epidemic. Here we will show how invasive garden ants use changes in the chemical profile of fatally infected colony members to detect infections before they become transmissible. Directed by these “sickness cues”, nest mates then engage in a multicomponent behaviour that destroys the infected individual along with the pathogen. This behaviour is so effective that the pathogen is no longer able to grow, and as such, cannot cause any secondary infections. By using chemical cues expressed in the early stages of infection, the ants can safely eradicate the pathogen without the risk of contracting the disease themselves. Thus, this behaviour will substantially reduce the risk of an epidemic and the impact infections can have on colony fitness.

Microclimatic preferences for waste management in leaf-cutting ants

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Leaf-cutting ants build complex underground nests, consisting of chambers in which they rear brood and symbiotic fungus, and separate chambers in which they dispose of their colony's waste. It is known that ants choose suitable temperature and humidity values inside chambers to rear brood and fungus, thus ensuring colony survival. Colony waste, composed of exhausted or pathogenically infected fungus pieces and dead ants, poses a potential health risk to the colony, and workers should choose carefully where to deposit this material. In binary choice experiments, we tested whether leaf-cutting ants (*Atta laevigata*) show preferences regarding their abiotic underground environment, i.e., temperature, humidity and CO_2-concentration, for waste deposition. Temperature regimes in the experiments ranged from 15 to 30°C, humidity levels from 10 to 98% RH and CO_2-concentrations from atmospheric values to 10%. Workers preferred a dry (10-33% RH) and warm (25°C) microclimate for waste deposition but showed no preferences regarding the CO_2-concentration of the chamber air. It remains untested whether these preferred conditions help to control the growth of pathogenic fungi in the waste material. We argue that the discovered climatic preferences have also implications for leaf-cutting ant nest architecture, as ants should aim to excavate spaces for waste deposition that offer the preferred microclimatic conditions.

Defend and disinfect: a dual role for soldiers in a complex insect society

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It is thought that the primary function of soldier castes in insect societies is to protect colonies against macro-scale predators and parasites. By comparison, little attention has been paid to the role of soldiers against pathogenic microorganisms. This is a significant gap in knowledge because in non-hymenopteran societies, such as the eusocial termites, the first-evolved, and arguably most important sterile caste, is the soldier. Here, we investigate whether soldiers of the wood-eating termite, *Mastotermes darwiniensis*, play a significant role in social immunity. Firstly, we show that uninfected soldiers improve the survival of worker nestmates exposed to a lethal dose of a fungal pathogen, despite the fact that soldiers (unlike workers) cannot allogroom. Hazard ratios of pathogen-exposed workers decreased from 6.616 in infected groups to 4.376 and 2.786 in infected groups containing uninfected workers and soldiers, respectively. To explore the basis of soldier protection, we applied soldier-derived oral secretions to infected worker cuticle, resulting in significantly improved worker survival. Soldiers that contacted infected workers were also more active and produced secretions with significantly greater mass variance than soldiers exposed to uninfected workers. Significantly, we show that secretions produced by soldiers inhibit the growth of a broad range of microorganisms, including gram-negative and gram-positive bacteria, in addition to fungi. Our findings demonstrate that soldiers can contribute powerful antimicrobial factors to external immune defenses, effectively complementing behaviour-based protective activities provided by workers.

Isolation of fungus-infected ants: a spontaneous or a nestmate-driven behavior?

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The ability to recognize self from non-self is central to the functioning of many biological systems. As a correlate, the social isolation of diseased individuals appears as a primary prophylactic measure in insect societies. In particular, in the case of a fungus-infection, contaminated individuals become a latent sanitary risk for the colony due to post-mortem corpse sporulation. We investigated whether *Myrmica rubra* ants can detect and keep at bay nestmates that are infected by *Metarhizium* fungus even when spores have already penetrated the insect body and are no longer present over the cuticle. Dyadic encounters showed that workers were actually more aggressive towards fungus-infected nestmates from the 3rd post-infection day onwards. This suggests that sick ants bear health-dependent cues that can be detected by their nestmates. However, within the social context of the nest, these infected individuals were neither discriminated nor actively rejected by gatekeepers. Social isolation of diseased nestmates thus appears on the basis of their spontaneous avoidance of nest interior. We will consider how such avoidance behavior may result from fungus-induced changes in the ant's response to environmental stimuli such as light intensity and/or to social stimuli such as the presence of nestmates or the level home-range marking. Finally, we will discuss the functional value of spontaneous isolation of moribund individuals that appears as a simple and widespread strategy for insect societies to reduce their exposure to sanitary threats.

Bridging the gap between individual and social immunity in colony disease defence

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In addition to individual immunity, social insects have evolved collective defence strategies against pathogens, which consist of behavioural, physiological and organisational adaptations. The importance of social immunity in preventing disease transmission in insect societies is well known. However, we still lack a clear understanding of how collective defence mechanisms are regulated and how they interact with the other regulatory systems of the organism such us the individual immune system. Here we assess how individual immune factors shape the collective immune response to pathogens by inhibiting a key termite immune effector: gram-negative binding protein (tGNBP-2). tGNBP-2 triggers downstream innate immunity, in addition to cleaving fungal and bacterial pathogens directly in the external environment. We established mini-colonies of *Reticulitermes flavipes* inside planar arenas where we were able to observe and record hygienic behaviours (i.e. allogrooming and cannibalism) in near-natural nest conditions. Colonies were either treated with the tGNBP-2 inhibitor or a control solution, after which 5 focal workers were introduced into each colony. Focal workers were pre-exposed to either: 1) the fungal pathogen *Metharizium anisopliae* 2) the t-GNBP-2 inhibitor + *M. anisopliae* 3) a control solution. Colonies were recorded and the hygienic behaviours performed by nestmates towards focal termites were analysed. We examined the impact of tGNBP-2 inhibition on individual innate immune regulation, in addition to exploring the wider impact of immune suppression on collective social behavioural responses.

Benefits and costs of social immunisation in ants

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Social insects protect their colony from disease outbreaks by providing health care to pathogen-exposed group members. While these sanitary actions reduce the risk of infection of the exposed individuals, they bear the risk for helpers to contract the disease themselves. In the invasive garden ant, *Lasius neglectus*, care-giving workers often receive low-level infections from their contaminated nestmates, which typically do not cause disease but instead trigger an immune-stimulation. This "social immunisation" reduces the ants' susceptibility to the pathogen upon secondary challenge. In nature, ant colonies face a great diversity of pathogens. We therefore studied how broadly this social immunisation protects ant workers against pathogenic threats. We can document pathogen-specific effects and the occurrence of costs. We further find that the ants can adjust their behavior to benefit from the protective effects of social immunisation, while keeping costs at a minimum. The diversity of pathogenic challenges that an ant colony faces thus leads to a complex interplay between its members and its multiple pathogens.

Exocrine secretions: internalized medicine?

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Antimicrobial and toxic secretions in the environment of an organism play a major role in mediating the outcome of several host-parasite interactions. These compounds improve an organism's survival and manipulate the microbial community surrounding it. Although external immune defence in the form of such secretions can be found in many different taxa, such as frogs, earwigs, beetles and even humans, the extension of parasite defence to the environment is especially well documented for antimicrobials from exocrine glands in social insects. In this study we explore whether the ant *Camponotus floridanus* also potentially ingests antimicrobials from exocrine glands and uses them as internalized medicine. In a series of experiments ranging from behavioural observations to experimental manipulation and microbiological tests, we evaluate benefits and costs of ingesting antimicrobial active compounds from exocrine glands. With this, our study complements the growing list of studies that have shown self-medication with environment derived compounds in several different, distantly-related, insect taxa and adds to our understanding of parasite defence and resistance in social insects.

Preliminary data on immunity transmission in *Crematogaster scutellaris*.

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Many social insects show immunization, an acquired reduced susceptibility to a pathogen after a previouse exposure, and the capacity to transmit it to colony members both vertically (through reproduction) and orizontally (through social contacts). We studied immunization mechanisms in the ant *Crematogaster scutellaris* in response to the generalist entomopathogenic fungus *Metarhizium anisopliae*. Founder queens were exposed to a non-lethal dose of alive or dead spores in early spring 2015 and were allowed to establish colonies. We evaluated colonial success (queen survival and offspring produced) and tested worker mortality after infection with lethal doses of spores. Queens exposed to alive spores had a higher success in terms of survival and resistance of offspring to the infection. Exposure to dead spores did not increase success compared to controls. We replicated the experiment in 2016 with strengthed pathogen (reared on the target host) which induced a higher mortality in the alive-spore group than control and dead-spores groups. Differently to 2015, survived queens of alive-spore group had a similar colony foundation success but queens of the dead-spores group produced a higher number of offspring compared to both control and alive-spore groups. Preliminary results also indicate the existence of a trasmission of the immunity; specific experiments will be carried out to understand which pathway (horizontal and/or vertical) is involved in this phenomenon. Overall our results suggest that the trasmission of immunity between queens and workers plays an important role in the success of *C. scutellaris* colonies.

Organisational immunity in ants

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Social insects live in dense, highly interactive groups of closely related individuals. They are therefore highly vulnerable to disease and have evolved collective defences to prevent entry and spread of pathogens into colonies. Selection for disease control is believed to have influenced the social organisation of insect colonies, for example by favouring their spatial and social compartmentalisation, thus impeding pathogen spread and protecting the queen and young workers from outside pathogens (‘organisational immunity'). Here we tested quantitatively whether the structure of interaction networks does confer disease protection in colonies of the ant *Lasius niger*. We automatically tracked the movement and interactions of all colony members before and after exposing a subset of workers to the entomopathogenic fungus *Metarhizium brunneum*, and we determined the fungal load of all individuals one day after exposure. We then combined network analysis with epidemiological modelling to uncover the transmission dynamics of the pathogen and determine the infection risk of individuals depending on their age, social task, and position in the network. Our results provide evidence for organisational immunity, suggesting that both inherent colony organisation and changes in the interaction patterns of the ants upon pathogen exposure slow down disease spread within the colony.

The effect of a floral isoflavonoid on the microsporidian *Nosema bombi*

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Bumblebees provide essential pollination services, however there is evidence to suggest that these charismatic insects are in global decline. An increase in parasite prevalence has been suggested as a contributing factor towards the observed declines. Plant tissues synthesize secondary metabolites, these are translocated into floral rewards and have negative fitness impacts for bumblebees. Whilst the presence of these compounds in floral rewards seems paradoxical there is evidence to suggest some have antimicrobial properties. Here we show how Biochanin A an isoflavinoid found in *Trifolim pratense* reduces microsporidian parasite load in newly eclosed workers. By selectively incorporating plant metabolites into colony food resources bumblebees can actively reduce larval parasite loads. Our findings highlight that understanding floral biochemistry is essential for improving bumblebee fitness.

The effects of floral resources on the parasite prevalence of bumblebees and solitary bees.

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Disease and the loss of floral resources are known to be important factors in the decline of many social and solitary bees. Pathogen spillover from domesticated colonies has been documented in several common species and for at least some parasites, prevalence appears to vary interspecifically. Unfortunately, we still know very little about pathogen occurrence in the wider bee community or how this might be affected by the surrounding floral resources. Nutrition modulates resistance and tolerance to infection, but whether compositional variations in the floral resource affect the pathogen load of bees is not known. To investigate this, floral resource surveys were carried out across urban and rural habitats in East Sussex, UK. Bumblebees and solitary bees were sampled from each and screened for *Nosema bombi, N. ceranae, Crithidia bombi, Apicystis bombi*, deformed wing virus and other pathogens. The incidence of these is reported for the species sampled and comparisons are made across habitat types. The relationship between pathogens and floral resource availability is discussed.

Do juveniles prophylactically increase their immune response in presence of infected sibling?

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A major cost of group-living is it inherent risk of pathogen infection. To limit this risk, many group-living animals have developed the ability to prophylactically adapt their immune system to the presence of group members. However, it remains unclear whether such an adaption may also reflect the immune status of the group members. Here, we addressed this question in the European earwig, *Forficula auricularia*, an insect species with a relatively long but facultative family life. Specifically, we tested whether juveniles prophylactically increase their expression of behavioral immunity, i.e. self- and allo-grooming, as well as their investment into immune parameters, i.e. phenoloxidase activity and hemocyte concentration, in presence of an immune-challenged sibling. We thus set up groups of four related juveniles, either immune- or control-challenged one of them, and subsequently measured the behaviors of each juvenile, as well as its immune parameters 24 and 48 hours after the challenge. We predict a higher expression of behavioral immunity, as well as a higher investment into immune parameters in groups including an immune- compared to a control-challenged juvenile. Such results would emphasize that juveniles have evolved fine tuned mechanisms to simultaneously control the risk of pathogen transmission within a family and limit the costs of an investment into individual immunity.

Individual and collective defence behaviors of ants against bacterial infection of the colony

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Social insects have evolved sophisticated collective anti-pathogen defences and intensive health care toward pathogen-exposed group members. Currently, the understanding of collective defences of insect societies is mostly based on studies focusing on 'external infections' induced by fungal pathogens that penetrate through the insect cuticle, and some studies using bacterial pathogens, introduced via injection/septic injury. However, many pathogens enter their hosts via the oral route, through contaminated diet or via cannibalism. Especially in the social insects, pathogens may easily be further transmitted orally via food exchange between colony members (larval feeding, food regurgitation). We have established a laboratory system to induce bacterial infections in the Argentine ant by oral route and study the individual and collective disease defences of the ants against these infections. We found characteristic changes in both self- and allogrooming, which indicate early pathogen detection and likely reduce the risk of disease outbreak in the colony.

Antimicrobial potential of the fungus-growing termite symbiosis

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Termites of the Macrotermitinae subfamily maintain a monoculture of an obligate mutualistic Termitomyces fungus in their nest as their main food source. The termites forage for dead plant material and mix it with Termitomyces spores in their gut to create a substrate - the fungus comb - for fungus growth. This substrate is degraded by Termitomyces, which produces nodules filled with spores that are mixed with new forage. Through this process, the plant substrate is ultimately completely decomposed for fungal and termite nutrition. Despite the expected diversity of putative antagonistic and competitor fungi in the forage material, fungus combs are essentially free of any fungi other than Termitomyces. We investigated whether this is due to the production of antimicrobial metabolites in the termite gut and comb environments. Liquid chromatography-mass spectrometry (LCMS) analysis of combs suggests that Termitomyces and bacteria-derived antimicrobial compounds may contribute to maintaining clean fungus combs. In addition, RNAseq of the gut microbiota of colonies of two termite genera (Macrotermes and Odontotermes) revealed that a number of PKS and NRPS gene clusters associated with antimicrobial functions are expressed. Collectively, our findings suggest that the gut bacteria of fungus-growing termites contribute to pathogen control through the production of antimicrobial metabolites during gut passage of forage material, and that the chemical environment within fungus combs upholds axenic conditions.

Change in the immunocompetence of honey bee workers with senescence and behavioural task

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Aging in honey bee workers is associated with a temporal polyethism in the tasks performed both inside and outside the colony. As workers age, they transit from house bees to foragers. Such behavioral transition is linked to a progressive immunosenescence, that is, a gradual reduction of the individual immunocompetence with workers' age. Interestingly, honey bee workers can revert from foraging to nurse tasks depending on the requirements of their social environment. In such case, also the observed immunosenescence appears to be a reversible process. Despite these experimental evidences, so far a specific association between immune system activity and the peculiar task performed by workers has not been deeply investigated. The main goal of our study was to understand if bees of comparable age showed differences in their immune system, and if such differences were somehow connected with their specific behavioral tasks. We investigated the variation of the immune activity of Apis mellifera ligustica workers, focusing both on their different age and specific task performed. Through immune challenge with Escherichia coli, we analyzed the immunocompetence of 1, 2 and 3 weeks old workers, versus foragers (22-45 days old) and guard bees (13-21 days old). Guard bees patrol the entrance of the colony, inspecting and contacting incoming bees.. Our results confirmed a progressive decrease in individual worker immunocompetence with senescence. However, guard bees showed a comparable immunocompetence, showing a similar rate of bacterial clearance, with regards to their same-age control bees despite their specialized task. Research funds by Italian PRIN 2012

Social grooming in the honeybee *Apis mellifera*: efficiency, spatio-temporal occurrence and physiological correlates

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In order reduce the risk of disease and parasite transmission associated to social life, social insects have evolved a plethora of strategies which can reflect in individual and colonial adaptations, such socio-spatial compartmentalization of colony members, social grooming and production and/or use of antimicrobial molecules. Here we investigated some features of social grooming in the honeybee, focusing on its efficiency against *Varroa destructor*, its spatio-temporal occurrence and on two possible physiological correlates of allogroming workers, i.e. constitutive immunocompetence and expression of soluble olfactory proteins.

Contrasting findings suggest that social grooming is a permanent specialization, or, conversely, a age-dependent task. Here we found that the behaviour is performed by 3-15 days old bees which also perform the other tasks expected from same-age colony mates. Spatial analysis showed that social grooming is performed mainly in a small area opposite to the foraging dance area, therefore suggesting that it might mainly be directed at younger bees. However, despite the weak specialization, groomers are more efficient in removing the *Varroa destructor*, than control honeybees.

Limited difference were found with regard to the expression of olfactory proteins in grooming bees with respect to same-age non grooming nestmate, while on the basis of bacterial clearance immune-assays, we demonstrated that allogroomers are significantly more immunocompetent.

Our results suggest therefore that social groomers are not highly and permanently specialized, while conversely they rely on a higher constitutive immunocompetence, that might help in reducing the immune risks associated with this task.

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Using the radial-arm maze to demonstrate impaired spatial working memory in bees following acute pesticide exposure

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In modern agricultural landscapes insect pollinators are frequently exposed to pesticides, including neonicotinoids, which may negatively affect cognitive traits. One such trait, spatial working memory, is likely to be important in avoiding previously-visited flowers and other spatial tasks such as navigation. We investigated the effect of acute field-realistic and high thiamethoxam exposure on spatial working memory in a key pollinator, the bumblebee *Bombus terrestris*. We tested bees on an analogue of the radial-arm maze (RAM), an established method in rodents and other vertebrates for assessing spatial working memory. We first demonstrated that application of the RAM to bumblebees is viable by showing that untreated bees performed significantly better than chance simulations, even when stereotypical behaviour was accounted for. Subsequent exposure to an acute sub-lethal positive control dose of thiamethoxam caused bees to make more total spatial memory errors (revisits to previously drained flowers), make these errors earlier, and take longer to complete the task. Bees exposed to two different estimated field-realistic doses also showed impaired spatial working memory relative to unexposed bees in the form of more and earlier errors. These effects were small compared to the positive control dose and dependent on bee size. The sub-lethal effects on spatial working memory found here have important implications for bee populations, because traits that potentially impair foraging efficiency and homing may produce knock-on effects at the colony level. This is particularly relevant in light of the imminent review of current EU restrictions on neonicotinoid pesticide use on bee-attractive crops.

Why social bees are so vulnerable to environmental stressors?

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As demands for crops that rely on pollination continue to rise most pollinator populations are in significant or severe decline. The many factors contributing to this crisis have been widely discussed and a legion of stressors has been identified. Understanding why bees are critically vulnerable to such stressors, using a neuro-ecological integrative approach, is the first step for conserving these animals and the essential pollination services they provide. Here we argue that the social bee lifestyle is particularly cognitively challenging. Bees use different cognitive strategies to effectively navigate and find and handle flowers. Social and central place foraging may have driven the evolution of particularly well-developed learning, memory and navigation capacities. Therefore bees are especially vulnerable to any cognitive or brain damage. Different human induced stressors (such as pesticides, heavy metal pollutants, parasites and dietary limitations) alter pollinators' cognition through damage to specific neuronal pathways within the multi-sensory integration centres of the bee brain. In these stressful conditions, as weak individuals undertake the foraging duty, the colony is endangered. In a healthy hive it is normally the older bees that forage. But in stressed colonies young and weak individuals participate in foraging. Therefore when a colony is under chronic stress and most vulnerable, the foraging force is least well equipped to support it. We illustrate this idea with preliminary data on the impact of nutritional stress on navigation performances by bumblebees foraging on artificial flowers.

Elevated virulence of an emerging viral genotype as a driver of honeybee loss

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Emerging infectious diseases (EIDs) have contributed significantly to the current biodiversity crisis, leading to widespread epidemics and population loss. Due to genetic variation in pathogen virulence, a complete understanding of species decline requires accurate identification and characterization of EIDs. We explore this issue in the Western honeybee, where increasing mortality of populations in the Northern Hemisphere has caused major concern. Specifically, we investigate the importance of genetic identity of the main suspect in mortality, Deformed wing virus (DWV), in driving honeybee loss. Using laboratory experiments and a systematic field survey, we demonstrate that an emerging DWV genotype (DWV-B) is more virulent than the established DWV genotype (DWV-A) and is widespread in the landscape. Furthermore, we show in a simple model that colonies infected with DWV-B collapse sooner than colonies infected with DWV-A. We also identify potential for rapid DWV evolution by demonstrating extensive genome-wide recombination *in vivo*. The emergence of DWV-B in naïve honeybee populations or in novel pollinator species (including via recombination with DWV-A) could carry significant ecological and economic repercussions. Our findings emphasize that knowledge of pathogen genetic identity and diversity is critical to understanding drivers of species decline.

Pesticides found in pollen and nectar collected by honey bee (*Apis mellifera*) foragers in urban environments

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Nectar and pollen samples from commercial apiaries have been shown to contain pesticides in various concentrations and diversity. In contrast, it is not clear whether stationary colonies placed in an urban setting are exposed to more or fewer pesticides, and at what concentrations. Colonies in urban environments could either be exposed to fewer pesticides than those in agricultural settings if foragers have access to untreated wildflowers and gardens, or they could be exposed to more pesticides if they forage from home gardens treated with more pesticides at higher concentrations. In July 2014, we started collecting monthly nectar and pollen samples from multiple colonies in urban areas of CA, FL, MI and TX (N= 15 to 18 colonies per state, 70 colonies total) to determine the type and concentration of pesticides found in fresh nectar and pollen. We sampled nectar collected and stored by bees in cells 24-72 h prior to sampling, and collected pollen from hive entrance pollen traps engaged 1-2 days prior to sampling. All samples were stored in dry ice and sent to an independent USDA laboratory in Gastonia, NC, for pesticide residue analysis. Each sample was tested for 179 insecticides, herbicides and fungicides. Preliminary results indicate that in all locations, the prevalence and loads of the pesticides found were lower compared to those that have been reported in commercial colonies, suggesting that stationary honey bee colonies in urban environments are relatively “clean” in terms of inadvertent exposure to pesticides. We discuss some implications of these findings.

Nutritional basis of behaviors: does availability access to carbohydrate resources enhance the competitive ability of invasive ant species?

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Variation in nutrition during development can have huge consequence on behaviour, growth, and the competitive ability of ant species. Nutrition is also considered to be a key factor influencing the ecological success of many invasive ant species. To test how protein and carbohydrate availability influence behaviour, we manipulated the macronutrient content of diets provided to colonies of three of the world's most widespread invasive ant species: *Solenopsis geminata*, *Pheidole megacephala* and *Paratrechina longicornis*. Specifically, we considered how variation in nutrition during larval development and as adults, influenced both individual and colony-level behavioral attributes including aggression, exploratory and foraging rates, and vigilance / defense. All experiments compared the abilities of the invasive ant species against a native ant: *Iridomyrmex pallidus.* We interpret results relative to the three different levels of environmental influence imposed by these species in their exotic ranges.

Reproductive potential of workers impact their foraging preferences in honeybee *Apis mellifera*

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Worker larvae which develop in a queenless colony may end up as so-called rebel workers. These rebels, occurring naturally after swarming, are more queen-like in terms of reproductive potential than normal workers. The proximate factor that influences rebels' development is the absence of a queen during their larval feeding period, whereas the decreased relatedness between the old queen's offspring and the new post-swarming queen's offspring appears to be the ultimate factor responsible for the shift in resource reallocation to reproductive tissues in rebels. Several authors suggested that reproductive potential of honeybee workers can impact their lifespan and non-reproductive division of labour including foraging preferences (nectar or pollen collection). To test this, we performed an experiment in which we compared foraging preferences of normal workers (lower reproductive potential) and rebels (higher reproductive potential). Our results showed that rebels live longer than normal workers ($P < 0.001$), but there are no differences in number of foraging bees in both tested groups ($P = 0.1123$). Rebels display foraging preference towards nectar, whereas normal workers show preference towards pollen ($P = 0.0304$). Rebels also bring larger volumes of nectar into the nest ($P < 0.001$), and that their nectar has higher concentration of sugar than nectar brought by normal workers ($P < 0.001$). However, there was no difference in pollen loads brought to the nest by rebels and normal workers ($P = 0.2038$). Our study confirmed that workers with different reproductive potential have different foraging preferences.

Ants display lower levels of rescue behaviour towards moribund nestmates

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Foraging ants are faced with a threat from co-occurring trap-building antlion larvae. In the event that an ant is captured by an antlion larva, nearby nestmates may exhibit risky rescue behaviour to save the captured ant from predation. Although the mechanism underlying the rescue behaviour remains a mystery, it is hypothesized that it is mediated by the action of the captured ant, which releases a facilitating pheromone. Here, the hypothesis was tested that the frequency of expression and characteristics of rescue behaviour exhibited by rescuer ants depend on the life expectancy of the victim ants. Decrease in rescue behaviour expression levels towards victim ants with low life expectancy may be adaptive at the colony level. In agreement with this hypothesis, results show that ants more frequently rescue their nestmates and perform more intensive rescues when the imperilled individuals have a higher life expectancy than when they have a lower life expectancy. Obtained results are interpreted as a decline in the rescue facilitation with decreasing life expectancy in ant victims. This rescue behaviour - life expectancy relationship may constitute a part of a broader social isolation syndrome of moribund ants, and/or reflect the high colony-level cost to saving individuals with low life expectancy.

Experimental study on the effect of abiotic factors in social insects' nest construction process

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How do individually “simple” and tiny insects build complex and sometimes meter-sized nests? Self-organization is a key theory that provides a framework to answer such puzzling question. In social insect colonies, plenty of interactions between individuals and between individuals and their environment occur at a local scale. These interactions may exert efficient positive and negative feedbacks that are a cornerstone of collective activity of self-organized systems.

Several theoretical models have been proposed during the last decades to account for nest construction in social insects. However, there is a scarcity of experimental studies that validate these theoretical models since the pioneering study of Bruinsma (1979, PhD thesis) on the construction of the royal chamber in the termite Macrotermes subhyalinus.

Here, we aim to gather experimental data to give new insights into the nest construction in ants and termites. We propose to assess the implication of abiotic factors (such as temperature and humidity) on individual behavior during the first steps of the construction process in the ant Lasius niger. Both factors vary inside ant nests, and they have been shown to modulate collective behaviors as corpse clustering dynamics in the ant Messor sancta (Challet et al 2005, Insectes Sociaux, 52: 309-315). Here we will assess the effect of these factors on the construction activity in L. niger and discuss the extension of these experiments on two mound-building termite species.

Effect of insecticide exposure on bee brain morphology and consequent impacts on behaviour

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Adult foraging bees come into contact with various neurotoxic pesticides in the environment. Neonicotinoid insecticides are considered to pose a high risk of exposure given the wide global use, and that residues are commonly found in the pollen and nectar of treated crops and nearby wildflowers. Thus, in social bees, residues in food brought back to the colony presents a potential route of exposure to developing brood. Whilst we know neonicotinoid residues can affect adult behaviour, we understand comparatively less as to whether it can cause trauma to developing tissues in brood and young workers, such as the brain, and how this might impact on behavioural tasks.

We investigated how chronic exposure to field-realistic levels (5ppb) of the neonicotinoid Imidacloprid affected the structural development of the brain of an important wild insect pollinator: the bumblebee *Bombus terrestris*. We tested whether exposure during either brood development or adulthood or both produced detectable effects on brain morphology. Using advances in high resolution microscopic computed tomography (micro-CT) and newly developed techniques in sample preparation and 3D image analysis (Smith *et al.* 2016 *Scientific Reports*), we measured the volume and shape of key brain structures for 76 individuals, and assessed how this correlated with individual performance in associative learning tests.

This work provides new insights into whether stress induced tissue trauma at key life-stages of an important social insect pollinator can affect adult phenotype. It is also important as impairment to behaviours that underpin foraging performance may lead to a reduced pollination service.

The interaction of environmental and social factors in the development of the larvae in ants *Myrmica rubra* L.

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The development of ant brood is under the influence of environmental temperature, as it is in the immature stages of solitary insects. But in ants, the social factors are also involved. The workers of M. rubra have been found to effectively control larval development during the photoperiodic induction and termination of diapause. When the ants that have been maintained under short-day conditions for several weeks feed larvae, they force them to enter diapause. On the contrary, the workers reactivated by 2-3 week exposure to long days or sampled just after hibernation (i.e. cold reactivation) stimulate the resumption of larval development even under short days. We have shown that in experimental conditions the intensity of tactile stimulation of larvae by nurse workers was greatest when physiologically active workers took care of diapausing larvae, i.e. during the termination of their diapause. The physiological condition of workers can influence the growth of larvae after overwintering as well. After hibernation, all larvae always pupate but the workers can modify their growth rate depending on whether the colonies are under long (22 h light per day) or short-day conditions (12 h). Under the latter photoperiod, larval growth rate is slightly slower than under the former. The differences are more pronounced at 21°C than at 24°C. In all experimental regimes, larval growth rate is significantly higher in groups with queens. Thus, the interaction of environmental and social factors may change the thermal reaction norm for larval development in M. rubra.

Interactions between gut bacterial community, landscape exposure and behaviour in honey bees

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The gut microbial community regulates a variety of functions in its animal hosts, including host health. However the relationship between the microbial community of the honey bee and its environment, key characteristics in the survival and evolution of this important ecological and economic pollinator, has yet to be investigated. We examine the relationship between environment exposure, both at the landscape and local level, and microbial community in the honey bee (*Apis mellifera*). Previous research has shown that a characteristic core set of bacteria inhabit the honey bee gut. We provide evidence that both the broad environment that bees are exposed to, and the local environment which workers are differentially exposed to due to the different tasks they perform, influence the relative abundance of some members of the microbial community. This includes known core bacterial taxa with functions in nutrition and health. Such environmental mediated changes in the microbial community likely also feedback into the bidirectional relationship between the gut and the brain (microbiota-gut-brain axis). Therefore the bacteria taxa we find to be differentially represented in bees performing different behaviours may also be key candidates in reinforcing behavioural division of labour.

Behavioural responses to pesticide exposure in bumblebees.

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Bees are amongst the most important groups of insect pollinators and provide a range of ecosystem services and functions that benefit human society and the natural environment. However, recent land use change, especially the move towards intensive agriculture, has been associated with reported population declines of social bees. One issue that has made the transition from the scientific arena firmly into the public consciousness is the effect of systemic pesticides, such as the neonicotinoids, that persist in many environments.

These neurotoxic pesticides are commonly found in the pollen and nectar of many flowering crops that are commonly visited by foraging workers of social bees. Exposure to these chemicals has been implicated as a driver of social bee losses via sub lethal impairment to foraging behaviour and subsequent effects on colony development and fitness - yet comprehensive evidence is still lacking to support this statement.

I present results from our laboratory and semi-field experiments demonstrating how, low-level, chronic exposure to neonicotinoids, over ecologically relevant timescales, affect key behavioural traits associated with foraging. We attempt to bridge the gap between lab and field experiments to elucidate the causative relationships between exposure and social insect behaviour. Finally, I discuss how our experimental work can be combined with modern molecular tools and sampling from wild populations, to ensure that our findings can be generalised to wild bumblebee populations.

Plasticity in daily behavioral rhythms of the ant *Camponotus rufipes*: the effect of food availability and caste affiliation

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In ants, timing between workers and their environment is essential for adaptive collective behavior. Although daily activity outside of ant nests is well studied in form of foraging rhythms, we lack information about behavioral activity rhythms inside the nest. To investigate the temporal organization of work in the ant *Camponotus rufipes*, we studied daily foraging activity in field colonies as well as behavioral activity of single workers in lab colonies. Field colonies were predominantly nocturnal, but always displayed some day activity. As daytime foraging occurred despite high temperatures and low humidity levels, the preferred night activity could be an adaptation to temporally restricted food access. Therefore, we investigated daily behavioral activities of single workers in lab colonies either under ad libitum feeding or daily pulses of food availability, either at day or nighttime. We analyzed 1656 hours of video recordings of subcolonies consisting of marked workers and brood under a light-dark-cycle and different feeding regimes. Under ad libitum feeding, foragers were active in the light and dark phase. However, foraging activity at colony level revealed the same dominant night activity as in the field. Under restricted food access, foragers synchronized their activity with food availability either at day or nighttime, exhibiting low activity levels. In contrast, nurses performed their tasks all around the clock with high activity levels independent of the feeding regime, likely to meet the needs of the brood. Thus, timing of daily behaviors in both castes was flexibly adapted to cope with environmental changes and task demands.

The ant-fungus mutualism of leaf-cutting ants: Microclimatic preferences to cultivate the symbiont

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Leaf-cutting ants excavate large, underground multi-chambered nests to rear brood and farm symbiotic fungus. To ensure colony survival, nests have to offer suitable microclimatic conditions. So far, studies showed that fungus is reared at warm and humid conditions. The carbon dioxide content of the nest atmosphere should also influence fungus farming as high concentrations (~4%), as they occur in deeper soil strata, hinder fungus growth. We evaluated for the first time the CO2 concentration preferences of leaf-cutting ants for fungus farming. In binary choice experiments, workers of Acromyrmex lundi could choose between chambers with different CO2 concentrations when relocating pieces of fungus. Concentrations offered ranged from atmospheric values ants would encounter aboveground, to 4%, as encountered in deep nests. Leaf-cutting ants avoided rearing fungus at those high concentrations, unless no other chambers with lower values were available. However, CO2-levels higher than atmospheric were not entirely avoided, as workers preferred chambers with values of 1%. The preferred CO2-levels corresponded to those measured in the shallow soil strata where nests of these species are found. It is possible, but yet untested, that concentrations of this magnitude are actually beneficial to the growth of the symbiont. The observed preferences for fungus rearing should also impact the workers decision where to enlarge their nests, ultimately influencing the nest architecture of this species.

Wolbachia as drivers of speciation in *Cardiocondyla obscurior*

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Wolbachia are common endosymbiotic bacteria in arthropods. Many strains of *Wolbachia* influence their hosts in various ways in order to increase their own transmission. Among these, reproductive manipulation is the best studied phenomenon, which includes feminization, male-killing, induction of parthenogenesis and hybrid breakdown through cytoplasmic incompatibility (CI). CI is the most common reproductive manipulation caused by *Wolbachia*. It occurs when *Wolbachia*-infected males mate with uninfected females or with females infected by a different *Wolbachia* strain. In general CI shows in reduced hatching success caused by sperm modification by *Wolbachia* during spermatogenesis, however it is as yet unknown how *Wolbachia* modify sperm. Here, we study hybrid incompatibility of *Cardiocondyla obscurior* ant colonies collected from Brazilian and Japan (Schrempf et al 2015). Queens from a Brazilian population mated with males from a Japanese population show decreased lifespan and fecundity, while the reciprocal crosses did well in comparison to intra-population crosses. This indicates reproductive manipulation by the Japanese wObs *Wolbachia* strain. We are in the process of making a detailed analysis of the *Wolbachia – C. obscurior* interaction. By treating Wolbachia infected colonies of *C. obscurior* with antibiotics (Rifampicin), and thus removing the *Wolbachia* population, we can directly test the role of *Wolbachia* in the cytoplasmic incompatibility observed between our two populations. We predict that Brazilian queens mated to Wolbachia-free Japanese males will experience no decreased lifespan or fecundity compared to allopatric matings.

The influence of carbon dioxide on the construction of ventilation turrets in leaf-cutting ants

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The giant underground nests of the leaf-cutting ants consist of a complex network of tunnels and chambers in which the ants cultivate a symbiotic fungus as their main food source. Due to fungal respiration the carbon dioxide levels inside the nest may reach up to 5% which is expected to inhibit fungus growth. In order to maintain low carbon dioxide levels in the underground, *Atta vollenweideri* nests rely on a wind-induced ventilation mechanism. In mature colonies several ventilation tunnels lead to the surface that allow the supply with fresh air (inflow) and the removal of carbon dioxide (outflow) from the underground nest. Ants actively enhance nest ventilation by constructing small turrets on top of central outflow tunnels. The structure of the turrets, i.e. number and size of turret openings is expected to be flexible, depending on the colony's ventilation demands.

We tested whether the construction of ventilation turrets is influenced by the carbon dioxide concentration in the nest by confronting a laboratory colony of *Atta vollenweideri* with airflow containing either 0%, 1% or 5% carbon dioxide and observing turret construction for 24h. Results show that while turret mass and height were the same for all series, number and size of turret openings significantly increased when carbon dioxide levels were elevated. We suspect, that under natural conditions, a higher number of turret openings and an increased turret aperture might promote the gas exchange with the environment by increasing the turret's open surface area.

Temperature variations affect the fighting ability of the invasive garden ant *Lasius neglectus* towards autochthonous species

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Temperature is one of the key factors affecting the structure of ant communities. The Middle Eastern invasive garden ant (*Lasius neglectus*) is known to tolerate a wide temperature range, and it can also survive winters with prolonged frost periods. This ability has favoured its rapid expansion towards central-northern Europe and continental Asia. In this study, we analyzed how temperature variations affect the competitive ability of this species towards two dominant Mediterranean ants, *Crematogaster scutellaris*, and *Tapinoma nigerrimum*.

Group battles were staged pitting groups of ten ants of different species against one another, at four different temperatures (15, 20, 25 and 30°C) and constant relative humidity (70%). Ants were observed at hourly intervals for up to 6 hours. Control tests without the presence of the opponent species were also run in parallel, to evaluate the effect of temperature per se.

Temperature variations differently affected the three test species. Both the survival and fighting ability of *L. neglectus* were negatively affected by high temperatures. On the contrary, the survival of the two autochthonous species was not affected by temperature changes, although their fighting ability declined with decreasing temperatures. In synthesis, these results suggests a negative synergistic effect of high temperature and interference competition on the survival of this invasive ant.

Tracking major endocrine regulatory pathways along queen's life cycle in the buff-tailed bumblebee, *Bombus terrestris*

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Bumblebees are outstanding models of primitive hymenopteran eusociality. Within their short lifespan, limited by the annual cycle of colonies in temperate climates, bumblebee queens undergo several dramatic shifts in life style and physiology, starting from a pre-hibernation solitary phase, through a solitary diapause to the social phase as colony foundress and queen. In insects, these transitions are accompanied by significant, hormonally regulated, changes in energy demand. However, in bumblebees, little is known about the endocrine signalling which regulates the alterations of physiological pathways throughout the queen life cycle. Therefore, we measured gene expression levels of major regulatory pathways across tissues, sexes, and life-stages in the buff-tailed bumblebee, *Bombus terrestris*. More specifically, we investigated: (1) Forkhead box protein O (FOXO) and insulin/insulin-like signalling (IIS), (2) Juvenile hormone (JH) pathways, and (3) Adipokinetic hormone (AKH) signalling. We show that queen diapause is regulated by FOXO, IIS and *Krüppel homolog 1* (*Kr-h1*), and that both JH and IIS have gonadotropic functions. Surprisingly, the endocrine regulation of the hypopharyngeal gland reveals a striking resemblance with the fat body across all investigated genes, sexes, and life stages.

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Always at your service, Your Majesty: Macrocyclic lactones elicit subordinate behavior in *Lasioglossum malachurum* workers

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One major precondition for the evolution of social living within insects was the establishment of specific communication systems, mainly in terms of chemical communication which is thought to mediate interactions of individuals, colony organization and the regulation of reproduction. In fact, chemical signals derived from queens are thought to affect the fertility and reproductive success of workers and are therefore key components to maintain the colonial success in social insects. Queen recognition by means of these fertility signals has been shown to occur in many social insects, however, their chemical structures have not yet been described in primitively eusocial halictine bees. In order to offer new insights into the evolution of sociality, the major aim of our study was to identify such queen recognition signals in the primitively eusocial halictine bee *Lasioglossum malachurum*.

We investigated cuticle surface odor bouquets of *L. malachurum* and performed bioassays to examine the function of cuticle compounds as queen recognition signals. In chemical analyses we found caste-specific differences in the cuticle surface odor bouquets mainly due to higher relative proportions of n-alkanes and macrocyclic lactones in queens. Moreover, in behavioral assays we observed a higher frequency of backing behavior shown by workers in worker-queen interactions compared to worker-worker interactions. A similar behavior was shown when workers were interacting with lactone-impregnated workers but not with alkane-treated ones nor with the control. Consequently a higher amount of macrocyclic lactones elicits subordinate behavior in workers and therefore functions as a queen recognition signal in *L. malachurum*.

The evolution of cuticular hydrocarbons in ants: The influence of inbreeding, parasitic lifestyle, caste and sex on chemical profiles

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Chemical communication is crucial for insect societies. Social parasites exploit insect societies and are selected to avoid host recognition. Here, we studied whether chemical strategies to avoid recognition evolve convergently, and whether they extend to queens, workers, and males alike. We analysed cuticular hydrocarbons of three ant social parasites and their related hosts to investigate whether a parasitic lifestyle selects for specific chemical traits. Slavemaker chemical profiles were characterized by shorter-chained hydrocarbons and a shift from methyl-branched alkanes to *n*-alkanes, presumably to reduce recognition cue quantity. These chemical shifts were consistent across-species, including independent origins of parasitism. Moreover, we detected caste differences across species, with workers carrying more and longer-chained hydrocarbons. Males showed no systematic chemical signal, despite their unique lifestyle. Our comprehensive study of cuticular hydrocarbons reveals how lifestyle-specific selection can result in convergent evolution of chemical traits.

In a second study on a facultatively inbreeding ant, we investigated how inbreeding affects cuticular hydrocarbon profiles. Genes underlying hydrocarbon production are thought to be under balancing selection. If so, inbreeding should result in a loss of chemical complexity. Indeed, cuticular hydrocarbon diversity strongly decreased with inbreeding in the ant *Hypoponera opacior*. Inbred workers exhibited lower numbers of hydrocarbons and less complex profiles. The association with inbreeding was particularly strong for n-alkanes. Our study demonstrates that odour profiles encode inbreeding information. Shifts in allocation strategies with inbreeding suggest that these ants can detect the inbreeding level of other individuals and use this information to adjust their reproductive strategy.

Queen pheromone signalling in the honey bee

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In complex animal societies in which there is a reproductive division of labour, the reproductively-active individuals sometimes produce signals that advertise their fertility. In eusocial insects, reproductive queens produce pheromones which can act to inhibit selfish worker reproduction, and suppress worker rearing of virgin queens. Queen pheromones have been suggested to represent an honest signal of queen fertility, and as such, the queen signal is expected to preferentially target the young nurse workers, as it is these workers that control the rearing of replacement queens, and also because these bees could develop functional ovaries and selfishly lay their own eggs.

Using an automated system for tracking the movements of and interactions between thousands of honeybees, we study two complimentary pathways for queen pheromone dissemination, namely (i) direct transmission via physical contact between the queen and retinue workers, and (ii) indirect transmission from queen to workers through deposition of pheromone onto the wax comb.

We show that both pathways maxmise the dissemination of pheromone. First, workers that have acquired a dose of pheromone by direct contact with the queen maximise onwards transmission by moving faster, and interacting more frequently and for longer with their nestmates. Second, by performing a self-avoiding walk, the queen increases the area of the comb that she covers with pheromone, which maximises the size of the audience that are indirectly informed of her presence. Last, using age-marked worker cohorts, we show that both mechanisms succeed in targeting the young nurse workers, in accordance with the prediction.

I smell where you walked - how chemical cues influence movement decisions in ants

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Interactions between animals are not restricted to direct encounters. Frequently, individuals detect and respond to cues unintentionally left by others. While such indirect cues have been intensely studied in predator-prey interactions, their role in interactions among competitors are little known to date. Ant communities are usually structured by aggressive interactions between competing species. Responding to cues of others should thus help to avoid competitors or discover food sources. In ants and other insects, such cues include chemical footprints, which they leave while walking.

We confronted ant colonies from four species with footprints of other colonies or species, and measured the workers' response. Moreover, we determined the chemical composition of footprints.

Lasius niger ants avoided footprints of non-nestmate conspecifics, and tended to avoid footprints of two further species. This shows that Lasius can distinguish nestmates from non-nestmates based on footprints alone. We suggest that they avoided encounters with competing colonies to reduce costly fights. In contrast, three other ant species approached allospecific footprints, which may represent eavesdropping to find resources discovered by others. The chemical composition of footprints was largely congruent to their species-specific cuticular hydrocarbon profiles. Footprint hydrocarbons left by single workers represented 1/170 to 1/64 of the quantity of their cuticular hydrocarbons.

We showed that chemical footprints represent an important cue for behavioral decisions in ants. The ability to identify and respond to chemical footprints may represent an important strategy for insects to cope with competing species or colonies in their habitat.

Oral exchange of growth hormones and proteins influences larval development in ants

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Social insects frequently engage in oral fluid exchange–trophallaxis–during which they pass the contents of their social stomach(s) between adults, and between adults and larvae. Although trophallaxis is normally considered a simple food-sharing mechanism, we hypothesized that endogenous components of this social fluid might underlie a novel means of communication. Through mass spectrometry and RNAseq analyses in the ant *Camponotus floridanus*, we found that trophallaxis fluid contains a contingent of specific proteins, hydrocarbons, microRNAs, and Juvenile Hormone III, an important insect growth regulator. When *C. floridanus* nursing workers' trophallaxis fluid was supplemented with juvenile hormone, the larvae they reared more often completed metamorphosis and became larger workers. Comparison of trophallaxis fluid proteins across social insect species revealed that many are regulators of growth and development. These results raise the possibility that trophallaxis is a communication mechanism that enables communal control of colony development.

Social parasites choose host nests by smell

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Nestmate 12. Recognition in social insects, the fundamental mechanism to protect colonies from intruders, is based on chemical signatures consisting of hydrocarbon blends. The complexity of these blends is huge and may have been favored by different selective pressures. Social parasites may select on host chemical signatures because they employ several chemical strategies to overcome nestmate recognition. A first step towards understanding the relationship between host-signature characteristics and parasite selective pressures on host chemical signature is understanding whether social parasites choose host nests depending on olfactory cues. The obligate social parasite wasp *Polistes atrimandibularis* lacks a worker caste and relies on that of its host, the free-living social paper wasp *Polistes biglumis*. Parasites invade host nests during the founding phase, when single foundresses (the only adults on colonies) deposit their signatures on nest surface. We tracked host-nest chemical signature by weekly collecting small fragments of nest-paper in 141 field host nests until 24 were invaded by social parasites. We then analysed the differences in chemical signature between host nests which were targeted or not by parasites. We found that parasites choose host nests based on odors: targeted nests had special proportions of hydrocarbons compared with non-targeted nests. This suggests that host nests bearing certain chemical signatures may be more likely to be parasitized and suffer parasite-driven fitness loss. These results support the hypothesis that social parasites may contribute to the evolution of host chemical signatures by imposing directional selection on the relative amount of its chemical components.

Aggression of foundresses as a potential mechanism for the maintenance of monogyny in the ant *Odontomachus hastatus*: what role for the integration of colonial and fertility cues?

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A major feature of social insects is the existence of reproductive division of labour where one individual reproduces and is assisted by a sterile worker caste. In ants, the number of reproductive individuals is a labile trait that has important consequences on social organisation. In earlier work, the issue of the number of queens was mostly addressed under an evolutionary perspective, much to the detriment of our understanding of the proximal mechanisms governing social organisation. One central question is to examine what mechanisms are used by colonies to control and limit the number of reproductives within nests. One privileged route to polygyny relies on the adoption of additional inseminated queens by established colonies. The maintenance of monogyny thus requires colonies to discriminate conspecifics potentially differing in their fertility status.

In the ant Odontomachus hastatus, we examined how monogynous colonies react to the introduction of conspecifics from different castes differing in their fertility and caste. Both field and lab experiments showed that resident workers were much more tolerant to alien workers and mature queens than to alien founding queens. This behavioural approach was combined with the characterisation of the cuticular profiles of individuals from different castes. The qualitative and quantitative differences in the chemical signatures of ants are likely to be responsible for the discrimination between castes and the elimination of potential competitive reproductives. Overall, our study suggests that resident workers combine both colonial and fertility cues to modulate their behavioural response.

Navigating in the dark: conflicting signals inside the nest of *Camponotus fellah* ants

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The spatial location of ants within the nest is a core mechanism in the formation of their complex social organization. However, the means by which ants navigate within the dark maze of the nest remain unknown: vision is impossible and gravitation gives directionality only in the vertical dimension. In our experiments, we examine the relative importance of signals that may guide ants as they move through the nest: surface pheromones, volatile pheromones and nest spatial orientation. We introduce colonies of individually-tagged Camponotus fellah ants with the task of larvae-retrieval in a dynamic nest-like set-up. We perform manipulations that create a conflict between signals, and compare their effect on ant navigation, analyzing parameters such as examinations and choices of different corridors, decision times and speed. Our results show that ants use chemical markings and spatial orientation in order to find their way through the nest. These chemical markings are efficiently present at bifurcations in the nest.

Could fungal infection shape discrimination abilities in ants?

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Social animals like ants, although seemingly well-protected against parasites and pathogens because of the protective nature of the social system, are exploited by several parasitic organisms. Not only the ants of the genus Myrmica seem to be particularly vulnerable to exploitation by different types of parasites, but in many Myrmica species, colonies frequently adopt unrelated gynes, which could be also viewed as temporary intraspecific social parasites. In our study, we investigated whether infection by the ectoparasitic fungus Rickia wasmannii changes the workers' threshold of acceptance of unrelated queens and alters the ant's behaviour towards non-nestmates in M. scabrinodis nests. For this purpose we performed aggression and adoption tests where we used workers and queens originating from infected and uninfected colonies. Our results demonstrated that fungal infection influences the interactions between workers and unrelated queens. Differences between infected and uninfected hosts might be consequences of changes in cuticular hydrocarbon profiles, although the precise mechanism responsible for that has to be still analysed. The current work was partially supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS - UEFISCDI, project number PN-II-RU-TE-2014-4-1930.

A conserved olfactory subsystem for social tasks in Hymenoptera? The basiconic sensilla subsystem of the hornet *Vespa velutina*

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Cuticular hydrocarbons (CHCs) are critical cues for intra- and interspecific interactions as they provide information about species membership, fertility status and colony membership. Although the chemical and behavioral aspects of nestmate discrimination are well documented in social Hymenoptera, much less is known about the detection and processing of these olfactory signals. In carpenter ants, previous work identified a female-specific olfactory subsystem potentially in charge of processing CHC information, through antennal detection by basiconic sensilla. It is still unclear however whether this specialized olfactory subsystem exists in other eusocial hymenopteran families which also actively apply nestmate discrimination. We thus assessed the existence of a possible homologous subsystem in Vespidae, a family in which eusociality appeared independently from ants. We analyzed the distribution of basiconic sensilla on the antenna of the hornet *Vespa velutina* and explored the projection pattern of its sensory neurons into the first olfactory processing center, the antennal lobe. The neurons from these sensilla project to a conspicuous cluster of small glomeruli with anatomical and immunoreactive features reminiscent of the ant CHC subsystem. Extracellular electrophysiological recordings further show that neurons within hornet basiconic sensilla respond preferentially to CHCs. These observations suggest that a CHC subsystem is conserved across distinct eusocial Hymenoptera families, and may have represented an ancestral preadaptation for elaborate intraspecific communication, potentially facilitating the multiple emergence of eusociality among Hymenoptera.

Evolution of queen pheromones and fertility cues in vespine wasps

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Vespinae wasps range from solitary, primitively eusocial to highly eusocial in which, there are a significant amount of workers able to reproduce even in the presence of the queen, leading to a conflict over male reproduction. The queen can assert their reproductive dominance by aggression and chemical communication through emitting fertility cues. Recently it has been discovered that specific queen-characteristic hydrocarbons signal the queen's presence and avoid worker reproduction across multiple lineages of social insects. Based on this broader evidence and in particular that in the common wasp *Vespula vulgaris*, several long-chain linear alkanes and 3-methyl-branched hydrocarbons were shown to act as queen signals, here, we carry out bioassays with another genus of highly eusocial Vespine wasps, the Saxon wasp *Dolichovespula saxonica.* We show that a blend of queen-characteristic hydrocarbons that are structurally related to the ones of the common wasp inhibit workers from reproducing. Furthermore, specific hydrocarbons showed to be highly abundant on the cuticle of queens in different species of *Vespula* genus. Overall, our results highlighted the central importance of cuticular hydrocarbons in the chemical communication of social insects and open the potential use of specific hydrocarbons in another species of social wasps in order to keep the reproductive division of labour inside the colony.

Comparison of substrate-borne vibrations in a paper wasp social parasite-host system: *Polistes sulcifer* and *P. dominula*

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Substrate-borne vibrations have been recently shown to play a significant role in social insects colony life. This holds true especially for *Polistes* paper wasps, in which vibrations have been hypothesized to be involved in caste determination (mechanical switch hypothesis) together with nutritional status (nutritional hypothesis). Interestingly, an obligate social parasitic species, *Polistes sulcifer*, performs an evident body shaking behaviour that likely produces vibrations. As obligate social parasites do not have the worker caste, unveiling the occurrence of a social parasite substrate-borne vibration produced by *P. sulcifer* in *P. dominula* parasitized colonies has the potential to provide new insights into the mechanical switch hypothesis. While vibrations produced by the host have been studied and suggested to be potentially related to larval feeding, nothing is known about the social parasite. Here, we describe and compare vibrations produced by both the parasite and the host species. Both produce low frequency vibrations but through different behaviors: *P. dominula* performs horizontal oscillations of the abdomen (abdominal wagging), while *P. sulcifer* emissions are caused by percussions of the abdomen against the nest (abdominal drumming). Moreover, parasite drumming is longer and has a higher dominant frequency compared to the host wagging, suggesting that it may represent an amplification of the host behaviour. Our results highlight this social parasite host system as a valuable model for research on substrate-borne vibrations and set the stage for future studies about the influence of vibrations on caste determination in *Polistes* paper wasps.

Tending without reward - chemical manipulation of ants by caterpillars?

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Ant societies are susceptible to infiltration by other insects despite their intricate chemical-based nestmate recognition system. Insect symbionts of ants exploit these bountiful insect societies through trickery and temptation. In a Panamanian forest, Thisbe irenea caterpillars feed on a specific host-plant. The plant and the caterpillars both secrete nutritious substances that Ectatomma ruidum ants consume. In return, these aggressive ants protect both organisms while guarding their food source. This study examines the aggressive response of the ants to disturbance when tending caterpillars. The immediate reaction of tending workers towards a threat was recorded under five different treatments that varied activity of the ant, the instar of the caterpillar, and its presence or absence. Surprisingly, workers were more protective of the 1st-3rdinstar caterpillars compared to the 4th-5th. The former have not yet developed specialized reward-yielding glands, thus eliminating one explanation why that the ants would provide this protection. Other possibilities are that the ants recognize the caterpillars as future providers of rewards, or that the ants do not recognize these caterpillars as caterpillars at all. To disentangle these two hypotheses, we used GC-MS analysis to compare the cuticular hydrocarbons (CHCs) of the caterpillars to the CHCs of the ants. Preliminary results show that the chemical profiles of the caterpillars and ant larvae are very similar, suggesting that caterpillars might suppress ant aggression and obtain protection by mimicking brood odour. This work illustrates how ants are vulnerable to deceptive caterpillars and how complex species interaction can be hidden behind seemingly straightforward symbioses.

Ants Discriminate Between Different Hydrocarbon Concentrations

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Social insects typically discriminate nestmates from non-nestmates using colony-specific blends of cuticular hydrocarbons, which may be considered as a chemical label. Within a species, the cuticular profile shows approximately the same qualitative set of compounds, although these differ quantitatively among colonies. Thus, the relative proportions of particular hydrocarbons may be higher in individuals of one colony compared to those of another (conspecific) colony. Social insects must perceive these differences in ratios in order to efficiently recognize non-nestmates. However, little is known about the underlying perceptual mechanisms. Here we investigated whether ants can discriminate between different doses of individual linear or methyl-branched hydrocarbons. We used the ant *Camponotus aethiops* as our study organism and differential conditioning of the *maxilla-labium* extension response as the experimental procedure, to test olfactory discrimination between two concentrations of the same compound (one rewarded and the other punished), using large (wide range, 1:100) and small differences (narrow range, 1:10) in hydrocarbon concentrations. Ants discriminated well between wide-range concentrations of the same compound, but showed asymmetric generalization between narrow-range concentrations. These results indicate that a certain differential in hydrocarbon concentration is essential for efficient discrimination.

Insect changelings: obligate social parasites produce brood with non-mimetic chemical signatures

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The wasp *Polistes atrimandibularis* is an obligate social parasite. It lacks a worker caste and instead relies on that of its host, the free-living social wasp *Polistes biglumis*, for rearing its own brood. *P. biglumis* has an efficient nestmate recognition system, largely based on colony-specific chemical signatures. While both host and parasite display species-specific signatures, female parasites avoid detection by chemically mimicking the colony they invade.

As a parasite takes over a nest, it progressively loses its species-specific compounds (alkenes). After a month, host and parasite chemical signatures are indistinguishable. Our goal was to investigate the chemical signatures of the social-parasite brood. Once a host foundress has been enslaved, she will accept and care for the parasite brood as if it were her own. This observation raises several questions. Does the parasite brood adopt a strategy for integrating itself into the host nest, just like adult parasites do? Does the parasite brood mimic the chemical signature of the host brood? We analyzed the chemical signatures of 160 preimaginal hosts and parasites (egg, larvae, and pupae) from 9 parasitized and non-parasitized nests (via GC-MS).

We found that the chemical signature of the parasite brood was relatively poor in hydrocarbons, especially in branched alkanes. Linear alkanes were also scarce and the most abundant compounds were alkenes (primarily nonacosene), which are absent in both host brood and adult signatures. These results suggest that parasite brood does not chemically mimic the hosts and escapes host detection in a different way than do adult parasites.

Theories of nestmate recognition

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The recent years have brought a radiation in the proximate explanations for nestmate recognition processes. Theories are diverse and range from invoking a template in the long-term memory, located in the higher brain, to sensillum-based decision processes. Here, I will try to summarize the different models and their testable hypotheses.

Physiological adaptations of *Cataglyphis* desert ants to severe heat conditions

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Ants can be found in nearly all terrestrial habitats, including some of the most inhospitable places of the world. As such, they are excellent model systems to study how organisms adapt to their environment. Among ants, hot desert *Cataglyphis* species are facing the most severe heat conditions. While they are foraging for insects dead from heat shock in plain day, the air temperature can reach up to 50°C. Despite several behavioral and biophysical studies, the physiological bases underlying their amazing tolerance to heat stress remain largely unknown. By combining thermal tolerance experiments, proteomic assays and quantitative real time PCR analyses, we have quantified and compared relevant heat-resistance metabolic processes among several species of *Cataglyphis* ants exposed to different heat conditions. Our results show how the regulation of these metabolic processes varies according to the severity of the thermal stress encountered in species' natural habitat.

The evolution of polyandry in the social Hymenoptera: A comparative investigation of the costs and benefits

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The evolution of mating and insemination of females by multiple males (polyandry) is a fundamental problem in behavioural ecology because it is generally considered to be costly to females**.** In many animals it can be explained by material benefits and facultative multiple mating is the most common state. In the social insects though, most of which are monandrous, many of the material benefits do not apply and polyandry is particularly puzzling as it reduces the high intracolonial relatedness that was key to their evolution. There are four leading explanations, why social insect queens may mate multiply: 1) obtain more sperm to produce more offspring; 2) improve the division of labour between their worker offspring; 3) increase the resistance to disease of their offspring colony; 4) dilute the effects of genetically incompatible matings. Using a comparative approach we investigate these hypothesis in 317 eusocial Hymenoptera species including ants, bees and wasps. Using phylogenetic independent contrasts to control for phylogenetic dependencies we find no association between mating multiple and the likely cost of mating (predation etc.), but significant relationships with colony size, division of labour, parasite load, and inbreeding. The results suggest that multiple benefits are important in the evolution of polyandry in social insects.

Continuous Morphological diversity of workers in the ant *Temnothorax nylanderi:* adaptive phenotypic plasticity or relaxed selection?

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Division of labour and colony efficiency in social insects are known to be improved by discrete morphological castes and subcastes. However, it is unknown whether colonies with continuous but limited worker polymorphism benefit from this diversity. In order to assess this question, we decreased worker size diversity in colonies of the ant *Temnothorax nylanderi*, a species where inter- and intra-colonial genetic diversity are low and where worker size diversity probably mainly originates from phenotypic plasticity. We reared manipulated and unmanipulated colonies in the laboratory. We found that the experimental decrease in worker diversity did not lower brood production, size diversity of newly produced workers, or colony performance when facing different challenges. Accordingly, worker diversity might not be adaptive and might result from temporal variations in environment. To test this, we reared unmanipulated colonies under normal or high temperature and compared the size diversity of laboratory-produced versus nature-produced workers. Surprisingly, diversity was higher in newly laboratory-produced workers for both temperature regimes, suggesting that worker size variation does not result from environmental variation. The higher diversity observed under constant laboratory conditions could result from the disruption of a colonial homeostasis. Hence, even though we did not find any fitness advantages to worker size diversity, the level of diversity appeared to be mainly under social control. We suggest that relaxed selective pressures on worker size due to social life might have been the primary force driving the evolution of worker size diversity in species with continuous but limited worker size variation.

Genome evolution of *Acromyrmex* inquiline social parasites

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A key challenge in evolutionary biology is to understand how new species succeed in adapting to novel ecological niches. We address this fundamental question in an ant lineage with multiple independent evolutionary transitions towards inquiline social parasitism, looking for convergent adaptations at the genome level.

The leaf-cutting ant genus *Acromyrmex* has at least four independent lineages of inquilines, each with their own unique host species and geographic distribution. All these inquilines have the so called phenotypic “inquiline syndrome”, but vary in degree of morphological specialization and loss of worker castes.

Using sequenced reference genomes of inquiline-host lineages from Panama and Uruguay, we established that social parasites evolved relatively recently (<6 MYA), and consistent with sympatric speciation as implied by Emery’s rule. This offers a rare opportunity to study genome-level dynamics during the early steps of convergent adaptive shifts to a novel social niche. By comparing the genomes of three *Acromyrmex* social parasites at different stages of evolutionary advancement and two hosts, we are in the process of retracing the extents to which morphological, caste-diversity, and odorant receptor traits underwent correlated evolutionary change, and will present the first results of this work.

The maintenance of defensive microbiomes and the avoidance of antibiotic resistance

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Can bacterial competition maintain microbiomes that offer hosts evolutionarily stable protection against a range of pathogenic threats? A common feature of such microbiomes is the presence of antibiotic producers, but a single strain producing a single antibiotic is unlikely to be effective long-term. How, then, do organisms reliant on the use of antibiotics tackle antagonistic pathogen defences, including the evolution of antibiotic resistance?

While microbiomes with defensive functions are ubiquitous from humans to insects to crop plants, these systems are rarely tractable in the lab. However, the fungus farming attine ants provide a unique social insect model system: We know that their cuticular microbiomes play a role in defence against both specialised and generalist pathogens, and that the ants live symbiotically with multiple species of antibiotic-producing actinobacteria – both vertically and horizontally transmitted. We also know that diversity of bacterial species generally correlates with diversity of antibiotics, and strongly suspect that the cuticular microbiomes have provided long-term protection against fungus garden pathogens and potentially also ant pathogens. Further, we have model predictions on how these microbiomes can be established by vertical transmission of native strains and selective recruitment of additional strains from the environment.

We tested these predictive models of host screening and microbiome assembly with particular focus on antibiotic resistance and production, combining insights from metagenome sequencing of cuticular microbiomes from across the attine phylogeny, sequenced genomes of several strains of vertically transmitted actinobacterial symbiont of *Acromyrmex*, and experimental tests of bacterial competition and invasion in antibiotic laden environments.

Gut bacterial transmission strategies in leaf-cutting ants

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The social Hymenoptera have distinct larval and adult stages separated by metamorphosis, which implies dramatic remodeling of external and internal anatomy during the pupal stage. This imposes challenges to gut symbionts as existing cultures are lost by lysis and may or may not need to be replaced. We traced the transmission of bacteria from egg to adult in the leafcutter ants *Acromyrmex echinatior* and *Atta cephalotes*, both important functional herbivores in the New World tropics. We found that larval and pupal guts of both species contained *Pseudomonas* and *Enterobacter* bacteria that are also found in fungus gardens, while adult workers did not or hardly have these bacteria, suggesting they are beneficial only for larval growth and development. Bacterial abundance remained similar across the developmental stages of *Acromyrmex*, but *Atta* pupae had vanishingly low prevalences whereas *Atta* workers had much more abundant and diverse gut communities than *Acromyrmex* workers. *Wolbachia* was the dominant bacterial species across developmental stages in *Acromyrmex*, but *Atta* lacked *Wolbachia* and had mostly Mollicutes bacteria in the adult worker guts. *Wolbachia* in *Acromyrmex* appeared to be transovarially transmitted similar to most solitary insects. However, the Mollicutes were absent in the eggs, present in the guts of some larvae, and disappeared from the pupal guts during metamorphosis, suggesting they are socially transmitted via fungus-feeding or trophallaxis. Our results reveal different strategies for bacterial OTU persistence within colonies and precisely define the developmental stages of bacterial acquisition and loss, which is important for clarifying their putative mutualistic functions.

Assortative mating limits gene flow between socially polymorphic ant species

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Research on hybridization between species provides insights into pre- and post-zygotic isolating mechanisms that drive speciation. The recent discovery and characterization of a hybrid zone between two closely related and socially polymorphic ant species offers the opportunity to investigate which reproductive barriers contribute to maintain species boundaries in this system. We investigated three mechanisms that may limit gene flow between species: temporal reproductive isolation, assortative mating and reduced viability of hybrids. We detected strong but incomplete assortative mating. Further, we showed that interspecies crosses produce viable offspring. These experimental results suggest that assortative mating limits gene flow between these two ant species, but does not result in complete reproductive isolation, a finding consistent with the genetic pattern observed.

Adaptation in populations of invasive fire ants

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Two closely related fire ant species Solenopsis richteri and Solenopsis invicta invaded the South-Eastern USA early in the last century. These species, which originate in South America, have become pests in introduced areas, severely affecting local wildlife, livestock and agriculture, causing millions of dollars in damages every year.

Samples of ants from different introduced and native populations were collected, sequenced and analyzed, allowing for extensive research to be carried out over processes that underlie the genetics of invasive populations, including founder effects, genetic drift and adaptation to a new environment.

We apply population genomic analyses to study the population genetics and structure of the fire ants. We create a population model of the demographic history of the native populations. Analyzing the samples genomic sequences against a closely related ant species, the thief ant S. fugas, we detect genomic loci that have experienced positive selection in the native range. We identify genes that were affected by selective pressures following the invasion into the USA by comparing sequence variation among ants from introduced and native populations. We predict the molecular biological functions of these genes and their possible evolutionary significance in the adaptation of the fire ants to the new environment.

Our study of two similar species that were independently introduced into the USA allows us to study parallel patterns of evolution and provides additional statistical support to our inferences. Together, these results provide insights into the molecular genetic basis of local adaptation in an invasive social insect.

Is there any link between foraging, defense and dispersion behaviors? Evidence from the candidate gene foraging

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Complex behaviors emerged from interactions between genes and environment. Understanding how genes can modify or be modified by behaviors is of primary importance to study species adaptation to their habitats. Social insect polymorphism is a good example of such behavioral adaptation to social environment constraint.

In this talk I will review, along with new results, the link between a candidate gene expression and three complex behaviors: foraging, defense and dispersion. Three different social insects were studied, *Pheidole pallidula* and *Solenopsis invicta* ants but also *Reticulitermes flavipes* termite. Our results show that the *foraging* gene controls defense and foraging activities and is negatively correlated to dispersion and aggressive behaviors in ants. Preliminary results on termites confirmed the evolutionary conservation of the *foraging* gene and its differential expressions according to castes. It also opens up opportunities for the study of the colonization and foundation processes of this xylophage pest. These results increase our knowledge of the role of *foraging* as a major player in regulating behavior responses to environmental change.

Bacterial but not protist gut microbiota align with ecological specialization in lower termites

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Lower termites form a mutualistic relationship with symbiotic protists and bacteria. These microorganisms facilitated the adaptation of termites to wood as a food-source. During the course of evolution, lower termites adapted to different lifestyles and diets, such as wood dwelling or foraging. The change from the ancestral wood dwelling to the derived foraging lifestyle coincides with higher exposure to pathogens as well as a more diverse diet. Since the microbial community is directly involved in substrate breakdown, it could contribute to the adaptation of its host to new substrates. If the microbes contributed to the adaptation of the host, we would expect different microbes in termite species with different lifestyles. We compared microbial communities across termites including three species that adhere to the ancestral life style of wood dwelling as well as two species adapted to foraging. We used 16S and 18S rRNA gene sequencing as well as metagenomic shotgun sequencing of termite guts to profile the associated microbial communities.

Soil mites (Oribatida) inhabiting red wood ant *Formica polyctena* nests and surrounding soil in SW Finland

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Red wood ants (Formicidae: *Formica*) build large, dense and long-lived nest mounds in boreal forest. Ant nests are also inhabited by other invertebrates, ant associates. The oribatid mite (Acari: Oribatida) fauna of nests is poorly known, both in terms of abundance and diversity. We compared the oribatid assemblages within and outside *Formica polyctena* nests in a Finnish oak forest. Altogether ten mounds were investigated and comparable samples were collected from adjacent soil 4 m apart. Our results showed that based on the distribution of 10,600 oribatid specimens and 74 identified species, the *F. polyctena* nest mounds were inhabited by an equally abundant and diverse oribatid fauna as the surrounding soil. For nest samples the abundance of specimens significantly correlated with diversity indicating that mounds form rich habitat patches with a diverse set of niches. Mound size had no effect on community composition. Moreover, the results revealed that the nest and soil habitats were predominantly occupied by different species resulting in dissimilarities in community composition; 34% of the species showed a preference for nest habitat and 50% for soil habitats, whereas 16% were indifferent with equal abundances in both habitats. Nonetheless, most of the species with a preference for nests are frequently found in various habitats in nature, and may be classified as facultative myrmecophiles that maintain populations also in ant nests. We conclude that ant nests host abundant and diverse oribatid assemblages, which differ from the fauna in adjacent soil, and wood ants are therefore important factors in maintaining biodiversity.

Horizontal transmission of the vertically inherited fungal symbiont of the leaf-cutting ant *Acromyrmex echinatior*.

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The leaf-cutting ant *Acromyrmex echinatior* lives in an obligate nutritional symbiosis with a fungal cultivar upon which it feeds. When virgin queens leave their natal colony to mate, they carry a small inoculum of their natal colony's symbiont in their infra-buccal pocket, which they use to initiate their own first garden. The fungus and the ants have chemical and behavioural adaptations to prevent contamination of clonal fungus gardens by foreign strains. However, molecular studies show a consistent lack of full congruence between ant and fungal phylogenies at fine scales, suggesting that horizontal transmission is relatively common. These exchanges have been predicted to happen at the colony founding stage and previous work showed that newly mated *Acromyrmex* queens deprived from their founding garden do accept a novel fungal strain in the laboratory before the emergence of the first workers. We tested whether such new acquisitions also occur in the field by removing incipient fungus gardens from founding nest cavities and creating orphaned fungus gardens by removing queens from nearby nests of similar age. We then followed the movements of the colour-marked queens and used microsatellite markers to track the movements of fungus gardens. This showed that fungus stealing occurs rather regularly and apparently without incompatibility problems in new partnerships. Our findings suggest an intriguing potential for overt conflicts between founding queens and their incipient gardens, as queens may try to steal gardens that better match their nesting habitat and fungus gardens may emit volatiles soliciting theft by other queens.

Pathogen spill-over among bees in Europe and Asia

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Though overwinter loss of honey bee (*Apis mellifera*) colonies has many causes, the last decade (2005/06 to 2015/16) has witnessed elevated colony mortality in Northern temperate regions. Pests and pathogens are often blamed, particularly Varroa (*Varroa destructor*) mites and associated viruses. Here I review my colleagues and my recent analysis of 14 positive single stranded RNA viruses associated with Varroa mites. Our empirical analysis at four pairs of locations around the world provides clear support for deformed wing virus (DWV) as the most prevalent and most abundant (per bee) virus correlated with Varroa mites. There is equivocal support for black queen cell virus (BQCV) also associated with Varroa. Other colleagues and I have screened wild bees, including bumble bees (*Bombus* spp.) and other genera in the families Andrenidae, Megachilidae and Apidae for the most prevalent viruses in honey bees. Going beyond our previously published results which demonstrated on-going transfer of DWV between honey bees and bumble bees in Great Britain (Fürst et al. 2014 Nature 506:364-366), we now find that DWV and BQCV are present in wild bees across Europe and Asia, sometimes at high prevalence. Other viruses can also be detected in wild bees. Open questions remain over the extent to which there is on-going transmission of these viruses between species, and the impact of viruses on novel host species.

Condition dependent effects of multi-host pathogens in a bumblebee

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Fast-evolving RNA viruses, known to cause colony losses in honeybees, can jump between host species threatening ecologically and economically important wild pollinators. Yet little is known about the impact of these multi-host viruses on wild pollinators. Acute bee paralysis virus (ABPV) and Slow bee paralysis virus (SBPV) are two RNA viruses that can cause high honeybee mortality in association with the *Varroa* mite, and both viruses are prevalent in a range of bumblebee species across the UK. Here, I present data from infection experiments of SBPV and ABPV on *Bombus terrestris audax* to show the impact of these common viruses on aspects of bumblebee health. SBPV-infection resulted in significant virulence under starvation conditions, with a 60% increase in the risk of death (infected bees die a median of 2 hours earlier than uninfected bees), whereas there was no effect under favourable conditions. High fat body increased longevity under starvation conditions. Our results demonstrate that a common honeybee and bumblebee pathogen, which may appear asymptomatic in field collections and under optimal lab-conditions, exerts a fitness cost on bumblebees under adverse conditions. These impacts may be greater in the wild under extra stresses of predation, competition and coinfection with other pathogens.

Viral diversity in ant communities

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Viruses are important infectious pathogens, regularly causing epidemics in societies, whether human or social insect. While research has made considerable progress in understanding viral infections in vertebrate societies, our knowledge in social insects is limited and almost exclusive to single viral infections in honey bees. In this study we explore the natural viral component communities, different virus species populating a single host species, of three host ant species across various sampling sites within Europe. Next generation sequencing allows us to catch the whole viral diversity within the sampled host populations and to disentangle real infective viruses from non-infective viruses. We will highlight conserved viral community patterns within single host species across our sampling sites and discuss differences in virus communities between invasive and native ant species. The powerful sequencing techniques at hand offer valuable insights into and a first step towards a basic understanding of complex ecological communities like the so far understudied multi-host multi-pathogen systems.

Bacteria living with long lived ant sperm

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Pathogen transmission between mating partners during copulation may be a major cost of sexual reproduction. Sexual diseases can impact the reproductive success of individuals, as they are likely to endanger the viability of the gametes, female fertility and in some cases, survival of mating partners. Besides infectious microorganisms that may be in contact with semen, mutualistic bacteria can also be found in the hemolymph, ovaries or testicular follicles of insects. These bacteria can play a role in protecting the host against other microorganisms, or by producing useful substances for the insect host (amino acids, sugars). Their presence in the reproductive organs and their potential role still remains ambiguous. The impact of bacteria in reproductive organs may be of particular interest in ants as queens store highly viable sperm for several years after a single mating episode.

Using the ARISA method, we investigated bacterial diversity in male seminal vesicles and accessory glands, and in virgin and mated queen bursa copulatrix and spermatheca of the ant Lasius niger. We determined the origin (male and/or female partner, external environment) of the bacteria in contact with sperm and their maintenance over time.

First evidence for sex-ratio biasing by *Wolbachia* in a social insect

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Social insect sex ratio and caste ratio are well-studied targets of evolutionary conflicts of interest, however the genetic architecture of these traits remain unknown. We carried out the first ant artificial selection study using the pharaoh ant *Monomorium pharaonis* to elucidate the genetic basis of caste. We intercrossed eight inbred lineages and then selected bidirectionally on colony investment in new queens versus workers (i.e. caste ratio). Across three generations of selection, we observed no evolutionary response for caste ratio, but the high and low selection lines did differ for sex ratio after the first generation. The initial inbred lineages varied in infection status by the maternally-transmitted bacterial endosymbiont *Wolbachia*, and we investigated its potential caste- and sex-biasing effects because *Wolbachia* is known to manipulate reproduction in other insects. Maternal lineages harboring *Wolbachia* increased in frequency in the two replicate high caste ratio lines and decreased in the two replicate low caste ratio lines. Overall, infected *Wolbachia* colonies showed a female-biased sex ratio 12% higher than non-infected colonies. While we did not find evidence for heritable genetic effects on caste, we do find evidence for the first case of sex ratio manipulation by *Wolbachia* in a social insect.

Ageing of *Myrmica scabrinodis* goes with heavier infection by *Rickia wasmannii*

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The myrmecophilous *Rickia wasmannii* (Ascomycota: Laboulbeniales) fungus infects different *Myrmica* (Hymenoptera: Formicidae) ant species. Their interactions have been started to be studied intensively in the last years. Results showed that the level of infection goes with heavier mortality of the host ants and the behaviour of heavily infected workers differed from the less infected ones. However, it was not checked whether there was any correlation between the age of the hosts and the level of their infection. In the case of positive correlation it can be hypothesised that not (only) the fungus but rather the age influenced the mortality and behaviour of the host ant workers. Here we examined the level of infection of three age categories of 540 *M. scabrinodis* workers to get answers to these problems. Our results showed that the older workers were covered by significantly more *R. wasmannii* thalli than the younger ones. The results highlight that it is important to think about both the age and the infection level of hosts in further setups at studying host-parasite interactions involving this fungal order. Furthermore, it is still a question whether there is time as a factor in the background of this positive correlation and/or that older workers perform different tasks than younger ones. By answering this question we could get a deeper understanding of the life of these peculiar fungi.

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