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The spatial arrangement of non-crop habitats affects the composition and dispersion of carabid beetles inhabiting an agroecosystem in Northern Italy

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The natural and seminatural components of agricultural landscapes play a crucial role in maintaining a high level of biodiversity. Being the Po Valley among the most human-dominated and intensively cultivated landscapes in Europe, we investigated the effect of non-crop habitats on carabid richness and composition. We also evaluated the role of tree rows as corridors for forest carabid dispersion. Carabids were sampled with 70 pitfall traps arranged in 35 sampling plots along three parallel transects (80, 100, and 140 m long) and encompassing five habitats: tree row, tree row edge, grassland, forest edge, and forest. We found 5,615 individuals belonging to 55 species. Despite the similarity in species richness, all the habitats investigated showed a peculiar and distinct species assemblage. The main distinction was between the "open habitat" cluster composed of grassland and tree row edge and the "forest" cluster composed of forest, tree row, and forest edge. We found that forest species can penetrate the grassland matrix up to 30 m from the forest edge and that a distance of no more than 60 m between tree row and forest can allow the passage of up to 50% of the forest species. Beyond this distance, the grassland matrix becomes a barrier, preventing them from reaching other suitable habitats. We confirmed that maintaining different natural habitats increases biodiversity significantly in an intensively cultivated agroecosystem. Moreover, we demonstrated the role of linear elements as a corridor and "stepping stones" for many forest species.

Keywords: agricultural landscape, biodiversity, dispersal, line transects, multi-model inference

In silico identification of neuropeptides and functional study of Adipokinetic hormones in Blattodea

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Termites and cockroaches belong to the Blattodea order of insects. As with other insect groups, they possess different neuropeptide classes for regulating various physiological processes. To date, only three neuropeptidomes have been characterized in Blattodea. Here, we aimed to carry out a comparative analysis of neuropeptides across the major termite lineages, including solitary cockroaches and subsocial wood roaches, focusing particularly on a comparative molecular analysis of adipokinetic hormones (AKH) and their receptors (AKHR). We then examine the potential role of AKH peptides in mediating immunity, by co-injecting peptides together with the entomopathogenic fungus, *Metarhizium anisopliae*. Our preliminary data show that co-application may significantly reduce termite survival. Mobilization of carbohydrates and lipids in the hemolymph of *Mastotermes darwiniensis* following peptide injection were also investigated. Our study represents the first to investigate the evolution of termite neuropeptides and their receptors as well as the metabolic role of AKH and its intersection with blattid host immune function. In addition, our study, provides a fresh perspective on the feasibility of using AKHs as potential candidates for biorational pest control agents against invasive termites and cockroaches.

Immune role of *Drosophila melanogaster* Kazal-type serine protease inhibitor CG14933

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Serine protease inhibitors (serpins) exhibit major regulatory functions in the proteolytic cascades of both arthropods and mammals. In *Drosophila melanogaster*, two of the major immune responses, the Toll pathway and the melanization cascade, are tightly regulated by serpins. Despite their known function in immunity, only a few of the 30 genes predicted to encode for serpins have been characterized, and knowledge about their exact mechanism in which they control the immune response to prevent excessive activation is still scarce. In this study, we investigate the immune role of the previously uncharacterized *Drosophila* putative serpin CG14933 containing Kazal domain. By using *CG14933SK1* flies, a mutant devoid of any *CG14933* expression, as well as RNAi knockdown and deficiency models, we show that flies lacking the gene show an increased susceptibility to *Pseudomonas entomophila* infection but not to any other infections that we tested. This clearly indicates an involvement of *CG14933* in *Drosophila* immunity, although *CG14933* itself is not induced by infection. We demonstrate that *CG14933* has no role in the regulation of major immune pathways and does not act as a neutralizer of bacterial proteases. Additionally, we found that in adult *CG14933SK1* flies, active phenoloxidase levels are significantly increased compared to wild type flies. We were able to rescue the sensitivity phenotype to *P. entomophila* infection by employing a double mutant devoid of both *CG14933* and *PP01*, the prophenoloxidase activated in early stages of infection. Moreover, by using tissue-specific RNAi-mediated silencing of *CG14933* we reveal a significant role of the gene in the fat body of *Drosophila*. Altogether, we propose that *CG14933* plays a role in negatively regulating the melanization response in *Drosophila*. Furthermore, we provide evidence of a possible link between excessive melanization and increased susceptibility to *P. entomophila* infection.

The ecology and distribution of several cryptic amphipod species from the *Gammarus fossarum* complex (Crustacea)

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The *G. fossarum* is a very common freshwater amphipod inhabiting mostly small streams in central and southeastern Europe. This morphospecies comprises dozens of old evolutionary lineages, some of which are dating back to the Miocene (ca 6 to 18 Ma) and their divergence reach up to 30 % on the COI barcoding fragment. Some of these lineages probably survived glacial ages in the Western Carpathians where they are currently in contact with each other, as well as with lineages spread also in other biogeographic regions. In our research, we confirmed syntopy of several lineages and studied their distribution and ecology on regional, local, as well as microhabitat scale in the Western Carpathians. We documented the existence of reproduction barrier among them by examination of pairing preferences and by analyses of both nuclear and mitochondrial markers. These lineages are therefore distinct biological species with independent evolutionary histories. The co-occurrence of these species is very common within streams in our study area. The coexistence seems stable in time, with no clear signs of different mesohabitat distribution (pools vs. riffles). It may be facilitated by niche differentiation, biotic interactions, or stochastic processes. Our regional scale dataset hints to different sensitivity to anthropogenic pressure, but we are not able to fully separate this effect from historical legacy of stream colonization. The preliminary results from a stable environment of a calcareous spring fen with confirmed presence of two *G. fossarum* lineages indicate differences in body size and carbon stable isotope composition but not in microhabitat preferences. It is possible that different body size and trophic ecology are important factors allowing the coexistence among at least some of these highly genetically divergent but morphologically similar gammarids.

Host-microbiota-pathogen interactions in the *Drosophila* gut

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Intestinal pathogens trigger immune system responses. The turn-on of the immune defenses reduces the pathogen bacteria loads and commensal bacteria diversity. The microbiota members that resist the Immune effectors provide essential health benefits to their host. Due to this, there is a growing interest in the basis of interactions between Host-microbiota-pathogens. However, the taxonomical complexity of commensal communities in mammals makes setting the basis of these interactions challenging. *Drosophila* gut harbors a low taxonomical diversity microbiota, and the Immune system response is mainly driven by the IMD pathway, making the fruit fly's intestine an optimal environment to investigate the basis of Host-microbiota-pathogens interplay. Here we sought the molecular mechanisms that make *Drosophila* commensals resilient to Antimicrobial peptides (AMPs). Overall, our preliminary data showed that *Drosophila* microbiota is more resistant to AMPs than intestinal pathogens, suggesting that the feature that leads microbiota to colonize the gut tissue and overcome the infections is the resistance to host AMPs.

How frequently are insects wounded in the wild? A case study using *Drosophila melanogaster*

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In order to counteract the potentially negative effect of parasites on host fitness, hosts have evolved a suite of mechanisms to reduce the probability and the impact of parasite infection. The insect cuticle is a tough, flexible, and waterproof barrier that functions as a protection against invasion by pathogens and parasites. However, it can still house diverse microbial communities and it may get damage due to intra-specific interactions, wear and tear, predators, or attack by parasitoid wasps. Damage on the cuticle might provide entry into the fly body for opportunistic pathogens, but very little is known about the frequency with which damage is found in wild insects. It has been reported that *Drosophila melanogaster* are often wounded in nature however, a systemic assessment of the frequency and degree of wounding in wild-collected flies is still lacking. Therefore, with this study we aim to examine the selection pressure of wounding on immunity in wild-collected *D. melanogaster*.

Host immunological control of gut microbiota during metamorphosis

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Antimicrobial peptides (AMPs) are defense molecules secreted by several organisms including insects. During metamorphosis, AMPs are synthesized massively in the insects' gut to eliminate possible pathogens although there is no infection. Thus, it is important to understand how holometabolous insects replace gut microbiota without causing resistance evolution. Here, we investigate 4 overexpressed AMP genes in greater wax moths' (*Galleria mellonella*) gut. We use the RNAi knockdown strategy to manipulate the synthesis of AMPs in different combinations. In this study, as preliminary steps, we produced dsRNAs for downregulating 4 AMP genes and designed efficient primers for detecting RNAi knockdown efficiency. As a result, 200bp long dsRNAs were produced for each gene. Additionally, all primer efficiencies were between acceptable range 90-110% (gallerimycin: 106%, lebocin: 105%, cecropin 2: 90%, lysozyme: 101%). All in all, essential steps for RNAi knockdown were completed.

Host and bacterial perspective of proteomic response during persistence infection

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Bacterial infections are dynamic processes, and it is becoming increasingly clear that bacterial infections can persist for the lifetime of an insect. This can be up to weeks or months in *D. melanogaster*, potentially increasing the opportunity and window for pathogen evolution. In *D. melanogaster*, many genes, including AMPs, continue to be transcriptionally upregulated between five- and seven-days post injection with bacteria and LPS. Given that AMPs can have a relatively short half-life it suggests active peptide translation and not peptides circulating in the haemolymph that had been translated days previously. Conversely, bacteria also employs different mechanisms to resist host AMPs by proteolytic degradation, sequestration and by the production of effector molecules or persist in a protected state inside the host. However, the reasons why pathogens are not cleared/how they manage to persist are poorly understood and characterised. To understand this, we will simultaneously examine the proteomic responses of hosts to persistent infections and the metabolic state of the persisting bacteria. Examining both host and pathogen proteomics gives an integrative approach to understand the molecular crosstalk in host-pathogen interaction.

Sex chromosome evolution in Madagascan chameleons of the genus *Furcifer* (Reptilia: Chamaeleonidae)

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Chameleons are well-known lizards with unique morphology and physiology, but their sex determination have remained poorly studied. We previously demonstrated with cytogenetic methods that the Madagascan chameleons of the genus *Furcifer* have distinct Z and W sex chromosomes. In this study, we micro-dissected and sequenced the sex chromosomes of *Furcifer oustaleti* and *Furcifer pardalis*, and we sequenced the genomes from both sexes of *Furcifer lateralis* and *Furcifer pardalis*, in order to identify the sex chromosome gene content. Our analysis showed that these chameleons co-opted for the role of sex chromosomes the same genomic region as viviparous mammals, lacertids, and geckos of the genus *Paroedura*, which makes these groups excellent model for studies of convergent and divergent evolution of sex chromosomes. Despite the notable heteromorphy and cytogenetic distinctiveness, the Z and W sex chromosomes share approximately 90% of their gene content. This finding demonstrates poor correlation of the degree of differentiation of sex chromosomes at the cytogenetic and sequence level. In addition, we compared the substitution rates of non-synonymous mutations normalized for the rate of synonymous mutations between autosomal, Z-specific, and pseudo-autosomal genes of *Furcifer* chameleons relative to outgroups with different sex determination systems. Our results show that there is no significant difference between these three categories of genes, which indicates the absence of a faster adaptive Z-effect in the chameleons of the genus *Furcifer* and that X/Z-specific genes might not always accumulate non-synonymous mutations faster than other parts of the genome.

Impact of population density on sexual selection in the red flour beetle

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The strength, form and direction of sexual selection have often been found to vary substantially within and among species. Theory predicts that demographic factors may play a critical role for understanding this intra- and inter-specific differences. One key demographic factor that often varies largely in space and time is population density, but we still know surprisingly little about its effect on mating behaviour and the strength of sexual selection. Therefore, we tested experimentally the effect of population density on mating behaviour in terms of the number of mating partners, mating duration and copulation attempts of males and females using the red flour beetle *Tribolium castaneum* as a model system. Specifically, we examined the effect of density by manipulating its two key parameters independently: group size and enclosure area. Hence, we were able to separate potential effects of the number of partners and competitors from effects of habitat area. Importantly, we contrasted the strength and direction of sexual selection across different densities using standardised metrics such as the opportunity for selection, the Bateman gradient and the Jones' index.

We predicted that density affects mating rate, which in turn may translate into changes of sperm competition intensity and female harassment by males. Moreover, we partitioned the variance in reproductive success to examine a potential shift from pre- to post-copulatory sexual selection under increasing density. Overall, our study aims at broadening our understanding of environment-dependent sexual selection by providing an experimental test allowing to infer a causal relationship between density and sexual selection.

Sexual dimorphism in *Drosophila* infection-induced defecation

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Benguettat et al. have characterized the role of defecation as a mode of bacterial clearance to help overcome intestinal infection using female *Drosophila*. Since the *Drosophila* intestine is sexually dimorphic, we aim to study how infection-induced defecation may vary between sexes and determine the role of defecation modulation in infection outcomes.

We use enteric infection with *Drosophila* natural pathogen *Pseudomonas entomophila*, which is more pathogenic to female flies than to male flies (upon ingestion). Flies are exposed to a colored solution containing *P. entomophila* and transferred to clear vials. Colored defecation spots are then counted for each experimental treatment and used as readouts for defecation rate comparison. The results demonstrate male bias in infection-induced defecation rates.

Then, we record the number of dead flies with altered defecation ability after exposure to *P. entomophila*. Male *Drosophila* mutants with reduced defecation ability strongly succumb to infection, while female *Drosophila* mutants with increased defecation ability show higher survival rates than the appropriate control.

Together, our findings demonstrate that defecation response differs between male and female *Drosophila*, affecting sexually dimorphic survival outcomes.

The evolution of termite sociality

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The phylogeny of the Blattodea boasts a wide degree of sociality spanning from solitary cockroaches to advanced ecosystem-dominating higher termite societies. The emergence of sociality in the termites was associated with the acquisition of a diverse range of social structures and differential expression of specific gene network. Previous work has found evidence for a caste-specific social defence system in termites leading to an immune system that may favour group over individual defence. While preliminary work suggests a correlation between social transitions and a reduction of immune gene family diversity, the lack of available high-quality termite genomes hampers complete knowledge of the true diversity of immune gene evolution across termite phylogeny. Here, we report on the sequencing and assembly of 50 high-quality long-read-based genomes and 180 caste- and sex-specific brain transcriptomes across major termite and cockroach sister-branch lineage. We investigate the diversity and evolutionary history of immune genes across genomes, and the potential gene networks that have evolved with the emergence of termite sociality.

Distinct genes are responsible for myrosinase activity in larvae and adults of the horseradish flea beetle, *Phyllotreta armoraciae*

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The horseradish flea beetle, *Phyllotreta armoraciae*, possesses an activated chemical defense that consists of sequestered glucosinolates and endogenous myrosinase enzymes capable of converting these glucosinolates to toxic isothiocyanates. Here, we identified three genes that encode enzymes with myrosinase activity in *P. armoraciae*. These myrosinase genes differ regarding their expression in larvae and adults: *PaMyr1* is expressed in adults, while *PaMyr2* is expressed in larvae. Compared to *PaMyr1* and *PaMyr2*, expression of *PaMyr3* was very low in all life stages. Using RNA interference, we confirmed that *PaMyr1* and *PaMyr2* are responsible for myrosinase activity in adults and larvae, respectively, whereas the function of *PaMyr3* is currently unknown. Predation experiments with *Harmonia axyridis* showed that *PaMyr2* expression correlates with larval survival, confirming that the glucosinolate-myrosinase system protects *P. armoraciae* larvae against generalist predators. Silencing *PaMyr2* expression additionally resulted in higher levels of sequestered glucosinolates, and decreased the proportion of hydrolysis products of ingested glucosinolates detected in the body, suggesting that endogenous myrosinase activity also mediates the turnover of sequestered glucosinolates in larvae. In summary, we show that different genes are responsible for myrosinase activity in *P. armoraciae* larvae and adults, suggesting that myrosinases have different ecological functions in these life stages.

Keywords: sequestration, glucosinolates, insect beta-glucosidase, two-component defense, ontogeny, tritrophic interaction

Adaptive evolution of inflammasome-related genes in amniotic vertebrates

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Inflammasomes are cytoplasmic multiprotein complexes comprising a sensor protein, inflammatory caspases, and in some (but not all) cases an adapter protein connecting the two. They form part of the innate immune system that triggers the activation of inflammatory cytokines, such as interleukin (IL)-1 β and IL-18, in response to infectious microbes and host-derived danger signals. To understand the adaptive evolution of animal inflammasome genes, we analyzed all gene families involved in inflammasome formation, including RIG-I-like receptors, NOD-like receptors, and caspases. The sequence data set covers all major clades of mammals, birds, and reptiles. In total, we detected 86 positively selected sites based on a consensus of three detection methods (MEME, FUBAR, and PAML). Aiming at these sites, we preliminarily describe the patterns of molecular convergent evolution acting in inflammasome-related genes between birds and mammals, as well as the relationships between phylogeny and molecular phenotype clustering. Our result provides the first prediction of interspecific differences and similarities in inflammasome activation potential. It will contribute to further research on the factors that shape the evolution of an optimal immune response.

The curse of the pharaoh in spore forming parasites

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The evolution of parasites, leading to increased or decreased virulence has attracted a significant amount attention from both theoretical as well as experimental biologists. This work began with the avirulence hypothesis which was eventually replaced as the prominent theory by the trade-off hypothesis upon which most of current understanding of virulence evolution is based. The trade-off hypothesis predicts the evolution of intermediate virulence, however there are many examples of evolution stable high virulence. One extension of the trade-off hypothesis proposed to explain some of these cases is the curse of the pharaoh hypothesis - also widely known as sit and wait hypothesis. Theoreticians have put forward multiple versions of this hypothesis, however, in laboratory settings-from natural conditions standpoint; testing these ideas and thereby understanding the dynamics of evolution is essential and currently under researched. We attempted to test the “curse of the pharaoh” hypothesis in host-parasite system. We established host parasite system using the red flour beetle *Tribolium castaneum* population (Cro1) as host and *Metarhizium spp.* as a panel of parasites. The “curse of the pharaoh” hypothesis revolves around the relationship between parasite virulence and durability in the external environment. A positive correlation would support the Curse of the Pharaoh hypothesis whereas a negative correlation would indicate a trade-off between environmental persistence and virulence. We hope to tackle the persistence of parasites in terms of elevated or dropped virulence by exposing parasites to the UV-rays as a proxy for environmental persistence.

Unveiling infection strategies of the entomopathogenic fungus *Metarhizium anisopliae* in the face of social immunity in the termite *Reticulitermes flavipes*

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Social insects such as termites boast ecosystem-dominating lifestyles, made possible by the evolution of permanent castes and an associated system of division of labour. A robust social immune system entailing collective defence against endogenous and exogenous threats, represents an important hallmark of advanced societies. Termites are no exception in that they display a broad repertoire of collective defences to protect colonies against infectious disease. To better understand mechanisms of social immunity in termites, it is important to unpick the dynamics of infection between invading pathogens and their hosts, including pathogen-driven responses in host immunity and other biosynthetic pathways, including those involved in cuticular chemistry which may play an impact role in communication. To achieve this, we present efforts to delete secondary metabolite genes using CRISPR/Cas9 in the entomopathogenic fungus *Metarhizium anisopliae*, a natural pathogen of termites. We focus on inactivating destruxins, which are key virulence factors synthesized by this fungus. We report on a method of transformation in this filamentous fungus, enabling us to successfully transform a GFP-containing DNA fragment in *M. anisopliae* blastospores. This reporter fungus paves the way for further knock-out steps as well as help us to visualize the infection stages in termites using fluorescence microscopy. We then discuss efforts to investigate changes in individual and social immunity as well as transcriptional and chemical changes following infection with modified fungal strains.

Cross-linked agarose-gelatin macro-beads: a new substrate to study bacterial biofilms

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One of the most significant challenges in treating bacterial infections is still posed by the resistance and tolerance of biofilms against the host immune response and antibiotic treatments. Biofilms underlie chronic infections. To study biofilm-forming bacteria, new methods are needed to tackle the specific characteristics of these survival strategists. Since biofilms are often surface-associated communities, beads for cultivation are used to study biofilms experimentally. To establish a more natural surface for biofilms, we present a new type of beads based on cross-linked mixture of agarose and gelatin that is sustainable in production and leads to more robust and meaningful results than artificial material. This protocol describes the production of the beads and their use in classical quantification methods such as CFU/mL counting and Crystal Violet staining, Light and Electron Microscopy, and time-kill assays with antimicrobials for different bacterial species. We have also studied the biofilm physiological response to different substrates by quantitative proteomics. The agarose-gelatin beads showed immense potential in all methods by being easy to handle and giving consistent results. Moreover, the efficiency in handling replication and the independent substrate and liquid phase manipulation can be promising.

The diversity of microsporidian parasites infecting divergent coexisting lineages of the *Gammarus fossarum* species complex in the Western Carpathians

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Microsporidia are a group of unicellular eukaryotic endoparasites often detected in high prevalences in freshwater amphipods, an ecologically important macrozoobenthos group. Microsporidians have different transmission strategies and diverse effects on the ecology and evolution of their hosts. Several of them are known to cause changes in reproduction, behavior, and sex ratio and affect the mortality of many gammarids. This includes the *Gammarus fossarum* complex, one of the most widespread and diverse amphipod species complexes in the Western Palearctic. Detailed analyses of *G. fossarum* populations across its European range revealed a particularly high diversity of divergent lineages in the Western Carpathians, which is in contrast with a rather uniform genetic structure in an adjacent westerly located region. Those lineages apparently represent distinct biological species, and their coexistence seems common in at least some streams in the regions of sympatry. However, their ecological differences and interactions are still poorly understood. Our study focuses on microsporidian lineage diversity and infection patterns with respect to host lineage identity within this species complex in the Western Carpathians. We screened over 2000 individuals of genetically characterized gammarid individuals for infection by PCR using microsporidian-specific primers targeting the small subunit rRNA. We documented not only the widespread presence of common microsporidian taxa known to infect *Gammarus* (genera *Nosema*, *Cucumispora*, and *Dictyocoela*), but also numerous rarer uncharacterized microsporidian clades. Some of them apparently represent potentially novel highly divergent clades, others are more related to (but distinct from) microsporidians already detected in other amphipod hosts. Additionally, our data reveal variation in the host lineage specificity in some common microsporidians in syntopic sites. These results add to knowledge about microsporidian diversity, infection patterns, and their potential role in ecological interactions and the coexistence of amphipod hosts.