# ESEB 2022 pre-symposium satellite event in evolutionary immunology & symbiosis

Date: 14.8. 2022

Time: starting from 10:00, ending at ca. 15:00

Venue: Charles University, Faculty of Science, Viničná 7, Prague; auditory B3 "Fotochemie" (50.07227797295658, 14.424178371130665), the building is ca 20 min. from the ESEB conference venue (<u>https://goo.gl/maps/7DJ1A4BqDmNJq2xU8</u>), the satellite event will end in time to get to the ESEB registration in time

Costs: there is no charge for this event

**Presentations: flash presentations only = 5 + 2 min.** (recommended 2-3 slides advertising the poster to be presented at the ESEB conference during the poster session – i.e. aiming to stress what is interesting about the topic of the poster without any details)

**Recording and streaming:** as requested by some of you, we will try to record the event and stream – we ask the presenters to indicate if they disagree with being recorded.

**Refreshments:** there will be a lunch break and one coffee pause; economy refreshments will be covered in the form of pizza for everyone (no need for a self-organised lunch)

**Further details:** <u>http://web.natur.cuni.cz/zoology/eei/eseb2022/</u> (further updates will be provided there before the meeting)</u>

### Programme:

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1	10:30	Martin Těšický	Different immune genes show variation in strength of positive selection: genomic approach in birds
2	10:37	Pascal Hablützel	High variability in MHC gene evolution among the adaptive radiations of cichlid fishes
3	10:44	Seraina Emilia Bracamonte	MHC evolution and expression patterns in the Neotropical Midas cichlid species complex
4	10:51	Tao Li	Adaptive evolution of inflammasome-related genes in amniotic vertebrates
5	10:58	Nadine Müller-Klein	Immune gene diversity as driver of tuberculosis susceptibility and resistance in wild meerkats
6	11:05	C. Rose McKeon	The genetic complexity of resistance to mycoinsecticides
7	11:12	Eva Lievens	Genotype-genotype interactions revealed in alga-virus coevolution
8	11:19	James Howie	Population genomics of disease transmissibility by apple psyllids and their coevolving endosymbionts
	11:30	LUNCH	
1	12:30	Lee Campbell	Influenza A virus infection induces differential expression of the duck TRIM gene repertoire
2	12:37	Srijan Seal	Evolution of divergent infection responses against single vs coinfecting pathogens
3	12:51	Teo Cereghetti	Natural selection on gene expression across invertebrate immune system
4	12:58	Nithya Kuttiyarthu Veetil	Evolution of immune responsiveness to mycoplasmal conjunctivitis in house finch populations
5	13:05	Venera Tyukmaeva	Susceptibility to trypanosomatid parasites in Drosophila melanogaster
6	13:12	Mehedi Hasan Fuad	Parasites inducing differential immune gene expression in sexuals and asexuals of gibel carp
7	13:19	Joana Sabino Pinto	Sexual selection and disease resistance: a case study in amphibians
	13:30	COFFEE BREAK	
1	14:00	Hanna Susi	Resistance variation and life-history trait correlations of Peronospora sparsa on three Rubus hosts
2	14:07	Sabrina Gattis	The effects of host age at exposure on Daphnia species infected by a yeast
3	14:14	Jéssica Teodoro-Paulo	Intraspecific variation in how a specialist herbivore affects plant defences
4	14:21	Salvador Espada-Hinojosa	Phenotypic plasticity of both partners in the giant ciliate Zoothamnium niveum thiotrophic mutualism
5	14:28	Barbora Thumsova	Climate warming triggers the emergence of native viruses in Iberian amphibians
6	14:35	Steven Fiddaman	Cryptic pseudogenization in penguin <i>TLR15</i> : an adaptive change or a ratchet to poor immune function?

### Abstracts

#### 10:30 Martin Těšický

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## Different immune genes show variation in strength of positive selection: genomic approach in birds

Theory predicts that during host-symbiont co-evolution, arms races diversify immune genes. However, it remains unclear if this holds true equally for all immune genes. Specifically, little is currently known about the frequency and strength of positive selection among different immune gene sets. Does the pattern of positive selection differ based on the gene's molecular function? Do membrane-bound proteins experience stronger selection than cytosolic ones? To answer these questions, we performed a comparative analysis of positive selection across immune genes in 50 avian genomes (ENSEMBL) and transcriptomes (sequenced by Illumina NextSeq after systemic LPS treatment). First, we compiled the list of immune genes based on human-chicken orthologs overlapping between databases AmiGO (GO: Immune system process) and Reactome (Immune system) enriched about chicken genes without clear human-chicken orthology. Then using three dN/dS-based methods (CODEML, FUBAR, MEME), we revealed positive selection in 340 protein-coding immune genes (≥ 1 positively selected site, PSS) and 240 (≥ 10 PSS) genes out of the total of 436 genes assessed. According to the Gene set enrichment analysis, strong effect of positive selection was revealed in cytokine receptor signalling, TLR signalling, NOD-like receptor signalling or RIG-I-like receptor signalling pathway but weak selection was found in genes involved in enzymatic activities. Our preliminary results also show that membrane-bound and extracellularly secreted immune genes are under stronger positive selection than those expressed in cytosol or nucleus. These findings support the hypothesis that immune genes whose products physically interact with pathogen structures are under stronger positive selection.

#### 10:37 Pascal Hablützel

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#### High variability in MHC gene evolution among the adaptive radiations of cichlid fishes

The adaptive radiations of cichlids are the result of the interplay between natural and sexual selection. This interplay is also reflected in their immune system. Among the immunity genes, the ones of the major histocompatibility complex (MHC) stand out for their high diversity. These pleiotropic genes play a central role in recognizing the antigens of parasites and (directly or indirectly) also in mate choice. They evolve following a birth and death process whereby new loci appear by duplication followed by gene loss at other loci. This process has led to high copy number variation of MHC class IIB genes in cichlids. The post-genomic era with the availability of hundreds of genomes has enabled the study of such complex gene families to unprecedented detail. In this study, I screened the genomes of more than 500 cichlid species for MHC class IIB genes. High levels of shared polymorphisms among different cichlid lineages indicate that most loci predate the contemporary adaptive radiations. Some lineages have a reduced MHC diversity and possibly even lost loci with classical MHC function, while others have a drastically expanded MHC locus repertoire. It remains unclear how trans-species polymorphisms are retained throughout the process of adaptive radiation. We may assume that selection for copy number variation is strong in many, but not in all cichlid lineages. This new exhaustive view on the evolution of MHC class IIB genes offers new opportunities to test hypotheses about the role of the MHC in parasite defense and mate choice in cichlids.

#### 10:44 Seraina Emilia Bracamonte

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#### MHC evolution and expression patterns in the Neotropical Midas cichlid species complex

Host-parasite interactions drive immunogenetic divergence of host populations across environments, fuelling diversification. MHC genes are key to the immune response in vertebrates and their great polymorphism is driven by parasite-mediated selection. MHC diversity varies among populations inhabiting contrasting environments and exposed to contrasting parasite communities. Differential expression of specific alleles could be a determinant factor for responding to parasites. We studied MHC class IIB divergence in the Nicaraguan Midas cichlid species complex, and associated expression patterns. The Midas cichlid repeatedly colonized crater lakes in which it has independently radiated to form parallel species flocks associated with contrasting habitats. Populations within and among lakes host parasite communities that differ in infection intensities, providing a setting for divergent selection to act. We found stable MHC IIB allelic divergence over years among Midas cichlid species inhabiting different lakes and habitats. However, allele pools fluctuated considerably within populations, providing evidence for ongoing selection dynamics. Additionally, we determined allele-specific MHC IIB expression among populations of the Midas cichlid. Identifying expression patterns of MHC alleles further elucidates the mechanisms underlying the host response to parasites and its role in shaping biodiversity.

#### 10:51 Tao Li

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#### Adaptive evolution of inflammasome-related genes in amniotic vertebrates

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 $\beta$  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.

#### 10:58 Nadine Müller-Klein

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#### Immune gene diversity as driver of tuberculosis susceptibility and resistance in wild meerkats

Pathogens of the *Mycobacterium Tuberculosis* complex, causing tuberculosis (TB), are highly transmissible and have a high capacity for between species transmission, making them a major global threat to a wide variety of mammal species. While the drivers of TB transmission and mortality are intensely studied in a small number of host systems, they remain generally poorly understood. TB infections caused by *M. suricattae* are characterized by prolonged latent periods, followed by rapid progression to terminal stages in meerkats (Suricata suricatta), and contribute significantly to mortality. Exposure to TB is high, but only a fraction of exposed individuals develop clinical TB, and the factors determining individual susceptibility, i.e. infection risk upon contact, and resistance, i.e. mortality risk upon infection, are not yet well understood. In this project, we leverage the exceptional long-term dataset the Kalahari-Meerkat-Project, which provides detailed behavioral, life history and health data for a wild meerkat population affected by TB, to investigate effect of Major Histocompatibility Complex genes on TB susceptibility and progression. We genotyped 1289 individuals, alive between 1993 and 2020, at the MHC-DRBexon 2 locus to test whether functional MHC diversity and/or specific alleles impact whether individuals develop clinical TB and how fast infections progress. This project will significantly add to our understanding of the genetic drivers of TB epidemiology, which can potentially be used to extrapolate the findings to other, less well studied mammal species affected by TB.

This project is funded by the German Research Foundation (DFG SO 428/15-1)

#### 11:05 C. Rose McKeon

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#### The genetic complexity of resistance to mycoinsecticides

When selection is strong and the genetic basis of adaptation involves a small number of genes, selective sweeps of beneficial alleles are common. However, most traits are complex, requiring the additive effect of multiple, even numerous, alleles. There is a long history of pest control in agriculture that exerts intense selection pressures on herbivorous insects. Despite concerted efforts to manage resistance evolution in pests, such strong selection has repeatedly brought about resistance. In many cases, resistance has been fast and straightforward to evolve, involving only one or two genes. Fungal entomopathogens, used in mycoinsecticides, provide arguably the most complex challenge widely available as a biopesticide. Although resistance to mycoinsecticides could involve numerous loci, this does not necessarily equate to resistance evolution being complex. The potential for resistance to evolve has already been shown; however, it is unclear how many loci are involved or whether there are gene-environment interactions that could affect resistance evolution. We use next-generation sequencing to identify genomic regions containing variants associated with resistance on an important axis of environmental variation - the crop plant on which the insect feeds. We pay special attention to the consistency with which such alleles reflect high fitness across environment. We also assess differential gene expression between susceptible and resistant genotypes. This work reveals the complexity and context specificity of the genetic architecture of fungal infection resistance in a globally invasive crop pest with an impressive, difficult to manage, track record of resistance evolution: the cotton bollworm, Helicoverpa armigera (Lepidoptera: Noctuidae).

#### 11:12 Eva Lievens

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#### Genotype-genotype interactions revealed in alga-virus coevolution

When microbial hosts and viruses coevolve, they often transition from arms race dynamics (escalating resistance and host range evolution) to fluctuating selection dynamics (stable coexistence of susceptible and resistant hosts caused by a growth-defense trade-off). However, this interpretation is commonly based on time shift experiments that use population-level samples for the virus partner. Consequently, it is uncertain whether the expanding host range of the population - i.e. the supposed arms race dynamics - actually occurred at the virus genotype level. We investigated this in the algal model Chlorella variabilis and its virus PBCV-1. The armsrace-to-fluctuating-selection pattern has been observed multiple times in this system, but always on the virus population level. We revealed the genotype level in two replicate communities from a Chlorella-virus coevolution experiment. For each community, we isolated ~40 virus genotypes (from 6 time points) and tested their ability to infect ~12 algal clones (from ~6 time points). We found that host range does expand at the virus genotype level, confirming 'gene for gene'-like GxG interactions and arms race dynamics. Interestingly, broad host range genotypes emerged and were maintained in one replicate community, but repeatedly appeared and disappeared in the other. We consider the mechanisms that could cause this difference, including populationdependent trade-offs and drift.

#### 11:19 James Howie

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# Population genomics of disease transmissibility by apple psyllids and their coevolving endosymbionts

Apple proliferation (AP) is a chronic disease of apple trees caused by the bacteria Candidatus Phytoplasma mali, which is primarily transmitted by two phloem feeding psyllid vectors, Cacopsylla picta and C. melanoneura. The extent of AP transmission arises from a complex interaction between Ca. P. mali, the psyllid insects, their primary and secondary endosymbionts, and the apple tree. Our research focuses especially on genetic variation in the psyllids and their endosymbionts associated with psyllid transmission efficiency and regional disease prevalence, based on field collections, field-laboratory experiments, and next- and third-generation sequencing. Populations of C. picta, the primary vector of Ca. P. mali across most of Europe, and C. melanoneura, an emerging vector in areas of North-Western Italy, were collected from North-Western and North-Eastern Italy. Isolines were established via single crossing with virgin females, and offspring sibling families were raised on experimental apple trees infected with Ca. P. mali. Quantitative PCR was used to determine disease uptake in individuals in each sibling family and, after high quality nuclear and mitochondrial psyllid and endosymbiont genomes were assembled, long- and short-read genomic sequencing of the sibling families was conducted. The resultant dataset allowed us to examine genetic differences associated with disease uptake after psyllids had fed on infected apple trees and with regions where co-evolution between Ca. P. mali and the psyllid vector was recent versus long established. Results provide insight into the coevolutionary population genomics of psyllids and their endosymbionts with respect to their effects on spreading an agriculturally important disease.

#### 12:30 Lee Campbell

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#### Influenza A virus infection induces differential expression of the duck TRIM gene repertoire

Tripartite motif (TRIM) genes are an ancient family of genes which has expanded throughout vertebrate evolution. Several TRIM proteins have demonstrated antiviral functions, including restricting viruses such as influenza A virus (IAV). Ducks are the natural host and reservoir of IAV and likely have evolved many strategies to control the virus. It is currently unknown which genes are present in the duck TRIM repertoire, and how many are involved in IAV resistance or restriction. To determine how many TRIM proteins were annotated in the duck we mined the NCBI protein database. We also assembled and interrogated a *de novo* duck transcriptome to search for any unannotated TRIM genes. We found 52 TRIM genes in the duck genome and transcriptome. To determine which TRIM genes responded to IAV infection, we examined the expression patterns of TRIM genes in ducks infected with a highly pathogenic H5N1 strain of IAV (VN1203) or a low pathogenic H5N2 strain (BC500). VN1203 replicates in lungs of infected birds while BC500 replicates in intestines. We determined that ducks infected with VN1203 differentially regulated 37 TRIM genes in lungs (20 upregulated and 17 downregulated). Ducks infected with BC500 had only 9 TRIM genes differentially expressed in lungs and 13 in intestines. Our analysis suggests several candidate TRIM proteins to test for antiviral function against influenza viruses.

#### 12:37 Srijan Seal

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#### Evolution of divergent infection responses against single vs coinfecting pathogens

In nature, hosts can face multiple pathogens simultaneously. While this might warrant activation of different immune mechanisms to counter mixed infections, an expansion of diverse immune arms together can collectively increase the costs of immunity, reducing the rate of adaptation against pathogens. However, a comparative experimental framework is missing. Here, we used several *Tribolium castaneum* beetle lines evolving against either a single or a combination of pathogens with divergent within-host growth dynamics— Fast-growing *Bacillus thuringiensis* (Bt); Slow-growing *Pseudomonas entomophila* (Pe); A combination of both (M). Although we began by imposing an equivalent selection pressure (~60% mortality in all lines), resistance could evolve most rapidly against Pe by overexpressing antimicrobial peptides and lysozyme (within 12-generations), whereas resistance against fast-growing Bt did not evolve yet, possibly because beetles could not invest more in fast-acting immunity relevant to Bt-clearance such as cytotoxic phenoloxidase. Expectedly, resistance evolution against M was delayed (~17-generations), perhaps due to increased costs of an extended immune repertoire. We did not find any reproductive costs in these lines, thereby suggesting the lack of immunity-reproduction trade-off.

#### 12:51 Teo Cereghetti

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#### Natural selection on gene expression across invertebrate immune system

A strong immune defence should evolve as a response to parasitism, which, however, can be constrained by associated trade-offs with other fitness-related traits. The form and strength of natural selection on immune activity are generally poorly understood. This is because of difficulties in examining which traits of a complex immune system are subject to selection under exposure to natural parasite communities. Here, we investigated natural selection on the gene expression of several components of the immune system of the freshwater snail *Lymnaea stagnalis* in a field experiment. We individually caged more than 200 snails in a pond for six weeks. We quantified snail fitness (i.e., total reproductive output) and immune activity at the gene expression level. We show that multiple components of the snail immune system were subject to natural selection. Positive directional selection predominated, especially for the components of non-self-recognition. However, also stabilizing selection on a few components of defence was observed. Interestingly, selection on the expression of some genes with similar functions varied (e.g., fibrinogen-related proteins). Our findings highlight the variation in natural selection on different components of the complex immune system and the power of multi-gene expression analysis to examine it.

#### 12:58 Nithya Kuttiyarthu Veetil

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#### Evolution of immune responsiveness to mycoplasmal conjunctivitis in house finch populations

Understanding mechanisms of host pathogen coevolutionary dynamics is a vital goal in evolutionary immunology. Yet, vertebrate model systems to investigate shifts in regulating host immune responses to pathogens are rare. In North America, a poultry pathogen Mycoplasma gallisepticum (MG) jumped in early 1990s to a new host, the house finch (Haemorhous mexicanus), causing millions of bird deaths due to severe conjunctivitis, a driving selective pressure across host populations. Here we report results of a common garden experiment using four different house finch populations (15 individuals per population) differing in their coevolutionary history with MG: a gradient from Virginia, where populations have interacted with MG for 20 years, to Hawaii, where populations are still naïve to MG. Birds were inoculated in conjunctiva with either control media or one of two MG isolates: the earliest known finch isolate (1994) or a more recently derived one (2013). We performed 3'-end transcriptomic sequencing on conjunctival tissues 3-days post-infection to reveal which genes are differentially expressed during infection and how populations differ in their interaction with differentially evolved MGs. In response to MG, we found upregulation of the IL17 pathway and downregulation of the IL12/IL23 pathway, suggesting significant role of  $\gamma\delta$  T cells and innate lymphoid cells in activating IL17-driven inflammation in conjunctiva. The same pathways were also differentially expressed between the host populations. We used RT-gPCR to confirm expression patterns of selected genes. Our results reveal a mechanism of house finch immunological interaction with MG that differs between host populations.

#### 13:05 Venera Tyukmaeva

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#### Susceptibility to trypanosomatid parasites in Drosophila melanogaster

Trypanosomatids are an extremely diverse group of single cell eukaryotic parasites infecting a wide range of animals and plants. Relatively recently, they have been discovered to be common parasites of insects in the wild, including different species of *Drosophila*. While *Drosophila melanogaster* has become an important model for evolution of innate immunity and host-parasite interaction, remarkably little is known about its genetic susceptibility to trypanosomatid infection. In the current study we investigate monoxenous trypanosomatids in several distantly related *Drosophila* species and demonstrate their genetic diversity. Further, we performed infection trials in fly strains from *Drosophila melanogaster* genetic reference panel to study variation in the host response to the infection and its effect on the flies fitness, assessed as survival, longevity and fecundity. We perform GWAS to unveil the genetic underpinnings of parasite susceptibility and plan to use RNAseq to complement our results with gene expression data.

#### 13:12 Mehedi Hasan Fuad

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#### Parasites inducing differential immune gene expression in sexuals and asexuals of gibel carp

Gibel carp (Carassius gibelio) is an invasive cyprinid species exhibiting a unique mixed reproductive strategy i.e., combining gynogenesis (asexual reproduction) and sexual reproduction. This fish species is parasitized by a wide range of metazoan parasites, and some differences in immunity were already documented between the two forms with different reproduction strategies. Differences in the effectiveness of the immune system and contrasting susceptibility to parasite infection may represent a potential mechanism facilitating the coexistence of asexual and sexual forms of gibel carp, thereby contributing to its invasive ability. In this study, the differential expression of genes related to immunity, activated by digenean species, was evaluated among three experimental groups: gynogenetic females, sexual females, and sexual males along with respective control groups. The sexual and asexual forms of gibel carp were infected by the larval stages (cercariae) of Diplostomum spathaceum (Digenea). Gynogenetic females were more infected than sexual specimens. Spleen was used as the target immune organ playing a role in innate and adaptive immune processes in fish. Based on the transcriptome profile analyses and extensive literature review, immune-related genes were selected, and their expression was quantified using RT-qPCR. We revealed, for the first time, the changes in immune gene expression profile involved in digenean infections in cyprinid fish. The contribution of different immune genes expression was highlighted as a potential mechanism contributing to the coexistence of sexual and asexual forms in nature.

#### 13:19 Joana Sabino Pinto

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#### Sexual selection and disease resistance: a case study in amphibians

Sexual selection is known to target disease resistance. This is seen in many vertebrate taxa where individuals tend to select mates based on traits related to disease resistance (e.g. MHC genotype). However, very little known about this mechanism in amphibians; this is particularly surprising as it is well established that there is strong sexual selection in many amphibian species. Currently, amphibians are experiencing massive population declines worldwide, with many of these declines being related to the emerging infectious disease chytridiomycosis. This disease is the result of an infection with fungi of the genus Batrachochytrium and has been associated with population declines of 501 amphibian species, out of which 90 went extinct and another 124 decreased in abundance by least 90%. Surprisingly, some populations thought to be driven to extinction by chytridiomycosis may be recovering, suggesting the evolution of resistance or tolerance. Hence, sexual selection for disease resistance could theoretically play a major role in population survival or recovery. We explore the potential role of sexual selection in such recovery.

#### 14:00 Hanna Susi

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# Resistance variation and life-history trait correlations of *Peronospora sparsa* on three *Rubus* hosts

Most pathogen species are generalists capable of infecting more than one host species. Tradeoffs in performance among different hosts are expected to limit the evolution of generalism. Despite the commonness of generalism, variation in resistance and transmission among host species have rarely been studied in wild. I studied field disease severity, transmission dynamics and host resistance variation of downy mildew pathogen Peronospora sparsa on its three host plants Rubus arcticus, R. chamaemorus, and R. saxatilis. In wild and cultivated populations of the three host species, disease severity varied by host species and whether the focal host species grew in shared habitat with other host species. The presence of *R. saxatilis* as an alternative host resulted lower disease severity in R. arcticus. To understand how resistance to P. sparsa varies among plant species and genotypes, an inoculation experiment was set up using P. sparsa strains from different locations and 20 genotypes of the three host species. Significant resistance variation was found among host genotypes but not among host species. When trade-offs for infectivity were tested, high infectivity in one species correlated with high infectivity in another host. However, when pathogen transmission-related life-history correlations were tested, a positive correlation was found in *R. arcticus* but not in *R. saxatilis*. The results suggest that host resistance may shape pathogen life-history evolution with epidemiological consequences in a multi-host pathogen.

#### 14:07 Sabrina Gattis

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#### The effects of host age at exposure on Daphnia species infected by a yeast

A major challenge of infectious disease epidemiology and evolutionary ecology is to predict disease outbreaks and how rapidly may spread in a population. It is widely accepted that host and parasite genetics, host susceptibility, parasite virulence, resource availability, and variation at the environmental and spatiotemporal levels play a vital role in the establishment of an epidemic. Nonetheless, factors related to host demography are often overlooked in epidemiological analysis, even though host's age is a key factor to take into consideration when determining the outcome of a parasite infection. Previous studies using the *Daphnia magna-Pasteuria ramosa* system have shown that juvenile Daphnia are more susceptible to infection than older ones. In this study, we investigated how general age effects are and whether they can be detected in different *Daphnia* hosts. By using three species of *Daphnia: D. magna, D. similis* and *D. curvirostris*, and their yeast pathogen *Metschnikowia bicuspidata*, we investigated the impact of host age on parasite-induced host mortality and on the relationship between pathogen virulence and transmission in different hosts. Age effects have been observed in all three *Daphnia* species. In general, *in D. magna* and *D. similis* the susceptibility to infection decreased with age while in *D. curvirostris* the opposite pattern has been observed. These results enhance

our knowledge on the relationship between pathogen virulence and transmission in different hosts, reinforces the epidemiological predictions behind it, and improves our response to an epidemic.

#### 14:14 Jéssica Teodoro-Paulo

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#### Intraspecific variation in how a specialist herbivore affects plant defences

Manipulation of host immune defences is a strategy used by herbivores to maximize their fitness by reducing plant defences. This seems to be ubiquitous for the specialist spider mite *Tetranychus evansi* – a model herbivore for host defence manipulation. Although the magnitude of induced tomato defences in response to feeding differs between different mite populations, it is not clear whether this variation is linked to fitness. To better understand this, we used four field populations and one laboratory outbred population created from controlled crosses of all four field populations, thus capturing natural, and assessed (i) their fecundity in the presence and absence of jasmonic acid (JA) plant defences, and (ii) induction of plant defences after mite feeding. We found that these populations varied in their fecundity in the presence of JA-defences and induced plant defences differently. We then investigated, using 59 inbred lines created from the outbred population, how much of this variability was due to genetic factors and whether it could be attributed to manipulation of JA-defences. We found that variation in fecundity among the *T. evansi* inbred lines has a genetic basis and is not influenced by JA-defences, indicating that factors other than these defences, influence fecundity. Indeed, JA-defences have a minimal impact on *T. evansi* performance most likely because all lines can adequately suppress them.

#### 14:21 Salvador Espada-Hinojosa

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## Phenotypic plasticity of both partners in the giant ciliate *Zoothamnium niveum* thiotrophic mutualism

The giant colonial ciliate Zoothamnium niveum is covered by a monolayer of sulfide-oxidizing bacterial ectosymbionts that show two different morphologies depending on their differential access to oxygen and sulfide for primary production. Bacteria close to the cilia beating oral part of the ciliate cells show larger sizes and are coccoid-shaped, while the ones observed in the aboral parts are smaller and rod-shaped. A suite of experiments under sulfide starvation in oxic seawater show diminishing populations of rod-shaped symbionts after 48 hours, and complete disappearance of the symbionts after 72h. In contrast, all symbionts are coccoid-shaped in sulfide supplemented oxic cultivations. Ciliate host colonies reproduction is through release of swarmers into the pelagial. Swarmers are covered by symbionts, ensuring the vertical transmission of the mutualism. Exposed to lack of sulfide dispersing swarmers completely lose the symbionts between 24 and 48h. Aposymbiotic swarmers fund aposymbiotic colonies with smaller size and short but wide shape due to higher number of zooids on each branch compared to symbiotic colonies. Because this morphology resembles the shape of the other, closely related Zoothamnium species, we interpret this profound change as the ancestral form. Conventional and well-established techniques such as fluorescence in situ hybridization and scanning electron microscopy already revealed aspects from both sides of this binary partnership. Easy access to Zoothamnium niveum in shallow marine waters and feasible manipulation in controlled experiments make this system a good candidate to employ additional approaches in order to enlighten mechanisms and processes from molecular to ecological and evolutionary levels.

#### 14:28 Barbora Thumsova

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#### Climate warming triggers the emergence of native viruses in Iberian amphibians

Emerging infectious diseases are important drivers of amphibian population declines and species extinctions worldwide One of the high-profile pathogens associated with these declines are viruses of the genus *Ranavirus*, casual agents of ranavirosis. Despite the increasing number of multihost epizootics, the origin of ranaviruses and the factors triggering their emergence remain generally undiscovered. We sequenced samples from amphibian carcasses collected in 15 never before studied incidents that occurred between 1988 and 2020 inclusive. We confirmed that two phylogenetically related viruses, the common midwife toad virus (CMTV) and Frog virus 3 (FV3) are responsible for mass mortalities in a wide range of amphibian taxa in Iberia. Our findings reveal that the highly virulent CMTV could be a European clade, with the Iberian Peninsula being the hotspot for CMTV diversity. The pattern of diversity found among CMTVs in Europe is consistent with spread by natural dispersal and we hypothesize that the Iberian Peninsula might contain the ancestral population of CMTVs that could have spread into the rest of Europe following the last ice age. Additionally, we find that climate warming could be triggering some of the ranavirosis outbreaks described here, supporting the endemic status of CMTVs in the Iberian Peninsula. Our study provides valuable new insight into the origin and emergence of the CMTV lineage and its spread throughout Europe.

#### 14:35 Steven Fiddaman

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### Cryptic pseudogenization in penguin *TLR15*: an adaptive change or a ratchet to poor immune function?

Pathogen-facing immune genes occupy a changing adaptive landscape; one in which pathogens continually evolve to evade recognition. Single-nucleotide polymorphisms and other small-scale variants are often the focus of investigations into host immune adaptation. At the other extreme, pseudogenization represents a rare but high-consequence means of honing the immune response and potentially avoiding immune-mediated pathology. However, our understanding of the mechanism of gene function erosion and eventual pseudogenization is poor, since the vast majority of pseudogenizations are fixed in the population. In this presentation, I will describe an instance of catching this process in action. In a large population study of *Eudyptes* (crested) penguins, we found several pseudogenized haplotypes of Toll-like receptor 15 (TLR15) – a TLR that recognizes fungal pathogens. Pseudogenized haplotypes were very common (>70%), but not universal, and there were infrequent intact haplotypes. However, functional analysis in an in vitro system revealed that the apparently 'intact' haplotypes were, in fact, non-functional. This contrasts to TLR15 from the closely related Emperor penguin and Northern fulmar, which were both fully functional. The apparently 'intact' *Eudyptes TLR15* haplotypes – devoid of premature stop codons - are therefore likely to be cryptic pseudogenes (genes which are capable of producing a non-functional protein product). TLR15 pseudogenization in penguins will be discussed in relation to immune function in a changing world; one in which the incidence of fungal pathogens, such as Aspergillus spp., will increase along with global temperatures. Will the ratchet of pseudogenization mean penguins are ill-prepared for future pathogen threats?