Charles University

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Department of Zoology

Study programe: Zoology

Summary of dissertation thesis

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Host-microbiota, pro-inflammatory immunity and physiological senescence in wild birds

Mikrobiota hostitele, zánětlivá imunitní odpověď a fyziologická senescence u volně žijících ptáků

Doctoral thesis

Supervisor: doc. RNDr. Michal Vinkler, Ph.D.

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List of papers

Host microbiota in the gastrointestinal tract

I. Kropáčková, L., **Těšický, M.**, Albrecht, T., Kubovčiak, J., Čížková, D., Tomášek, O., Martin, J-F., Bobek, L., Králová, T., Procházka, P. & Kreisinger, J. (2017). Codiversification of gastrointestinal microbiota and phylogeny in passerines is not explained by ecological divergence. *Molecular Ecology*, *26*(19), 5292–5304. doi: 10.1111/mec.14144 $IF_{2017} = 6.09$

II. Kubovčiak, J., Schmiedová, L., Albrecht, T., **Těšický, M.**, Tomášek, O., Kauzálová, T. & Kreisinger, J. (2022). Within-community variation of interspecific divergence patterns in passerine gut microbiota. *Ecology and Evolution*, 12:e9071. https://doi.org/10.1002/ece3.9071 $IF_{2022} = 2.77$

III. Schmiedová, L., Kreisinger, J., Kubovčiak, J., **Těšický, M.**, Martin, F.-P.J., Tomášek, O., Kauzálová, T., Sedláček, O. & Albrecht, T. (2023). Gut microbiota variation between climatic zones and due to migration strategy in passerine birds. *Frontiers in Microbiology*, 1–13. doi.org/10.3389/fmicb.2023.1080017 $IF_{2022} = 6.06$

IV. Kropáčková, L., Pechmanová, H., Vinkler, M., Svobodová, J., Velová, H., **Těšičký, M.**, Martin, J.-F. & Kreisinger, J. (2017). Variation between the oral and faecal microbiota in a free-living passerine bird, the great tit (*Parus major*). *PLoS ONE*, *12*(6). doi: 10.1371/journal.pone.01799450 $IF_{2017} = 2.77$

V. Schmiedová, L., Černá, K., Li, T., **Těšický, M.**, Kreisinger, J. & Vinkler, M.: Bacterial communities along parrot digestive and respiratory tracts: the effects of sample type, species and time *(submitted to International Microbiology*)

VI. **Těšický, M.**, Schmiedová, L., Krajzingrová, T., Gómez Samblás, M.M., Bauerová, P., Kreisinger, J. & Vinkler, M.: Nearly (?) sterile avian egg a passerine bird (*submitted to FEMS Microbiology Ecology*)

Evolution of innate immunity receptor diversity

VII. Vinkler, M., Fiddaman, S.R., **Těšický, M.**, O'Connor, E.A., Savage A.E, Lenz, T.L., Smith, A.L., Kaufman, J., Bolnick, D., Davies, CH.L., Dedic, N., Flies, A.S., Gómez Samblás, M. M., Henschen, A., Novák, K., Palomar, G., Raven, N., Samake, K., Slade, J., Veetil, N. K., Voukali, E., Höglund, J., Richardson, D.S. & Westerdahl, H.: Understanding the evolution of immune genes in vertebrates (*submitted to Journal of Evolutionary Biology*)

VIII. **Těšický, M.**, Velová, H., Novotný, M., Kreisinger, J., Beneš, V. & Vinkler, M. (2020). Positive selection and convergent evolution shape molecular phenotypic traits of innate immunity receptors in tits (Paridae). *Molecular Ecology*, (April), 3056–3070. doi: 10.1111/mec.15547 $IF_{2020} = 6.19$

IX. Włodarczyk, R., **Těšický, M.**, Vinkler M., Novotný, M., Remisiewicz, M., Janiszewski, T. & Minias, P.: Divergent evolution drives high toll-like receptor (*TLR*) diversity in passerine birds: buntings and finches (*submitted to Developmental and Comparative Immunology*)

X. Krchlíková, V., Hron, T., **Těšický, M**., Li, T., Hejnar, J., Vinkler, M., & Elleder, D. (2021). Repeated *MDA5* gene loss in birds: An evolutionary perspective. *Viruses*, *13*(11), 1–12. doi: 10.3390/v13112131 $IF_{2021} = 5.81$

XI. Krchlíková, V., Hron, T., **Těšický, M.**, Li, T., Ungrová, L., Hejnar, J., Vinkler, M. & Elleder, D. (2023). Dynamic evolution of avian RNA virus sensors: Repeated loss of *RIG-I* and *RIPLET*. *Viruses, (1)*15, 1-15. doi.org/10.3390/v15010003 $IF_{2022} = 5.81$

XII. Divín, D., Goméz Samblas, M., Kuttiyarthu Veetil, N., Voukali, E., Świderská, Z., Krajzingrová, T., **Těšický, M.**, Beneš, V., Elleder, D., Bartoš, O. & Vinkler, M. (2022). Cannabinoid receptor loss makes parrots susceptible to neuroinflammation. *Proceedings of the Royal Society B*, 289. doi: 10.1098/rspb.2022.1941 $IF_{2022} = 5.53$

Physiological senescence

XIII. **Těšický, M.**, Krajzingrová, T., Eliáš, J., Velová, H., Svobodová, J., Bauerová, P., Albrecht, T. & Vinkler, M. (2022). Inter-annual repeatability and age-dependent changes in plasma testosterone levels in a longitudinally monitored free-living passerine bird. *Oecologia*, *198*(1), 53–66. doi: 10.1007/s00442-021-05077-5 $IF_{2022} = 3.23$

XIV. **Těšický, M.**, Krajzingrová, T., Świderská, Z., Syslová, K., Bílková, B., Eliáš, J., Velová, H., Svobodová, J., Bauerová, P., Albrecht, T. & Vinkler, M. (2021). Longitudinal evidence for immunosenescence and inflammaging in free-living great tits. *Experimental Gerontology*, *154*, 111527. doi: https://doi.org/10.1016/j.exger.2021.111527 IF_{2021} = 4.03

XV. Bauerová, P., Krajzingrová, T., **Těšický, M**., Velová, H., Hraníček, J., Musil, S., Svobodová, J., Albrecht, T. & Vinkler, M. (2020). Longitudinally monitored lifetime changes in blood heavy metal concentrations and their health effects in urban birds. *Science of the Total Environment*, *723*. doi: 10.1016/j.scitotenv.2020.138002 IF_{2020} = 7.96

Other papers (not part of the thesis)

Palomar, G., Dudek, K., Wielstra, B., Jockusch, E. L., Vinkler, M., Arntzen, J. W., Ficetola, G.F., Matsunami, M., Waldman, B., **Těšický, M.**, Zieliński, P & Babik, W. (2021). Molecular Evolution of Antigen-Processing Genes in Salamanders: Do They Coevolve with MHC Class I Genes? *Genome Biology and Evolution*, *13*(2), 1–15. doi: 10.1093/gbe/evaa259

IF_{2021} = 3.41

Veetil, N.K., Oliveira, H.C., Gómez Samblás, M.M., Divín, D., Melepat, B., Voukali, E., Świderska, Z., Krajzingrová, T., **Těšický, M.**, Beneš, V., Madsen, O. & Vinkler, M. Application of the 3' mRNA transcriptomic sequencing (QuantSeq) for identification of differential gene expression during neuroinflammation in the zebra finch (*submitted to Immune Network)*

Abstract

Triggered by microbial ligands, inflammation serves as a "double-edged sword" to fight infections on the one hand, but on the other hand causing tissue damage due to oxidative stress if it is dysregulated. For example, chronic inflammation can contribute to *inflammaging*, which is now widely regarded as one of the causes of ageing. In my interdisciplinary dissertation, my colleagues and I investigated three interrelated aspects of inflammation, using an evolutionary framework and various free-living birds as models: (1) ecological and evolutionary determinants of gut microbiota (GM) composition and diversity, a driver of wild bird immunity, (2) diversity in immune genes affecting inflammatory responses in wild birds and (3) inflammation-related physiological senescence in a free-living passerine bird, the great tit (*Parus major*). Firstly, using *16S rRNA* gene metabarcoding, we revealed high intra- and interspecific variation in passerine gut microbiota dominated by the major phyla Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. Although in mammals GM depends strongly on host phylogeny and diet, in birds we found only moderate effects of phylogeny and very limited effects of host geography and ecology on GM composition. While microbiota diverged between the upper and lower gastrointestinal tracts (GIT), the microbiota of the adjacent tissues in the lower GIT was very similar, which is consistent with the relatively homogeneous GIT morphology in passerines and parrots. To understand the initial recruitment of GM and the mechanisms behind it in birds, we further investigated the microbiome of avian egg content and developing embryos in the great tit. We found that bird eggs were nearly sterile before hatching, suggesting that GM must predominantly form only after hatching in passerines. All this shows that GM is different in passerines compared to mammals and highlights that results derived from mammalian GM studies cannot be generally translated to passerines. Secondly, we developed a broadly applicable methodological pipeline to detect adaptive variation in protein-coding genes based on structural evolutionary bioinformatics, positive selection and adaptive convergence testing. Adopting this pipeline, we revealed that receptors of innate immunity, such as Toll-like (*TLRs*) and RIG-like receptors (*RLRs*) were highly variable in their ligand-binding regions in birds and much of their variation evolved adaptively. For the first time, we detected multiple gene losses in viral-sensing *RLRs*, retinoic acid-inducible gene I (*RIG-I*) and melanoma differentiation-associated protein 5 (*MDA5*) across the avian phylogeny. We further discovered the intriguing gene loss of cannabinoid receptor 2 (*CNR2*) in parrots, which negatively regulates inflammation and whose loss led to increased brain neuroinflammation. Thirdly, using longitudinally monitored great tits, we demonstrated senescence in multiple physiological traits that differ in their lifetime trajectories Chronic inflammation, which was positively associated with general oxidative stress damage, increased with ageing, documenting for the first time *inflammaging* in birds. Conversely, induced cellular inflammatory responses underwent bell-curved trajectories, consistent with *immunosenescence*. The same polynomial age-related trend in male plasma testosterone documented *hormonal senescence*. In contrast, levels of heavy metals in blood were largely independent of age, showing that bird blood can be used to monitor current heavy metal exposure even if the age is not known. All this suggests that small passerines undergo similar age-related changes as mammals.

Abstrakt

Mikrobiálními ligandy vyvolaný zánět je klíčový imunologický proces, který na jedné straně zajišťuje obranyschopnost proti infekčním onemocněním, ale jeho dysregulace může vést také k oxidačnímu poškození tkání. K tomu dochází např. během chronického zánětu, který může způsobovat tzv. *inflammaeging*, o němž se nyní uvažuje jako o možné příčině stárnutí. Ve své multidisciplinární disertaci jsem za využití evolučně-komparativních přístupů a ptáků jako modelové skupiny zkoumal tři vzájemně propojené aspekty zánětu: (1) evoluční a ekologické determinanty vnitro- a mezidruhové variability střevní mikrobioty (SM), které ovlivňují evoluci ptačí imunity, (2) genetickou diverzitu imunitních genů ovlivňující zánětlivou imunitní odpověď u ptáků a (3) stárnutí ve fyziologických znacích spojených s tzv. *inflammagingem* na modelu volně žijícího pěvce, sýkory koňadry (*Parus major*). (1) Pomocí sekvenování bakteriální *16S rRNA* jsme zjistili značnou vnitro- i mezidruhovou diverzitu ve složení SM u pěvců a dominanci taxononů z kmenů Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. V porovnání se savčí SM, které je do velké míry determinována fylogenezí hostitele a jeho stravou, naše výsledky u pěvců ukazují pouze na středně silný vliv fylogeneze hostitele a velmi slabý vliv ekologických faktorů (potrava a geografie). Zatímco složení mikrobioty mezi dolním a horním trávicím traktem bylo značně divergované, SM se mezi různými úseky střeva příliš nelišila, což také dobře koresponduje s málo morfologicky diverzifikovaným dolním trávícím traktem létavých ptáků (zejména pak u pěvců a papoušků). Sekvenování mikrobiomu ptačího vejce u sýkory koňadry odhalilo, že jejich vejce je *téměř sterilní* a že ke kolonizaci ptačího střeva mikrobiotou dochází u pěvců až po vylíhnutí. Naše výsledky tak ukazují, že složení SM se výrazně liší mezi savci a pěvci a že výsledky získané studiem savčí mikrobioty nejsou univerzálně přenositelné na ptáky. (2) Abychom detekovali adaptivní variabilitu v imunitních protein-kódujících genech, vyvinuli jsme univerzální metodologický postup využívající detekci pozitivní selekce, adaptivní konvergence a dalších evolučně-bioinformatických metod. Vazebná místa receptorů vrozené imunity, konkrétně Toll-like receptorů (*TLRs*) a RIG-like receptorů (*RLRs*) byla u ptáků velmi variabilní a většina této variability byla predikovaná jako adaptivní. Jako první jsme u ptáků detekovali mnohočetné ztráty v RLRs, konkrétně v genech *RIG-I* (retinoic acid-inducible gene I**)** a *MDA5* (melanoma differentiation-associated protein 5**)**. Podobně také gen pro kanabinoidní receptor 2 (*CNR2*) byl opakovaně ztracen u papoušků, což by mohlo vysvětlit, proč papoušci častěji trpí neurozánětem v mozku a různými neurodegenerativními onemocněními. (3) Naše výsledky u sýkory koňadry ukázaly na fyziologickou senescenci v mnoha sledovaných znacích, jejichž trajektorie se nicméně během stárnutí značně lišila. Zatímco chronický zánět spolu s oxidační poškozením tkáním postupně lineárně narůstal s věkem, což poprvé u ptáků jednoznačně ukazuje na vliv *inflamagingu* během stárnutí, experimentálně vyvolaná buněčně-zánětlivá odpověď měla polynomickou závislost na věku, což ukazuje na *immunosenescenci* ve funkční imunitní odpovědi. Podobnou polynomickou závislost na věku měla také hladina samčího testosteronu, což naznačuje na hormonální senescenci. Naopak hladina těžkých kovů v krvi prakticky narůstala během stárnutí u opakovaně odchycených jedinců, což naznačuje, že stanovení těžkých kovů v krvi se může využít pro biomonitoring environmentální kontaminace těžkými kovy i v situacích, neznáme-li přesně věk odchycených ptáků. Naše výsledky tak ukazují, že u malých pěvců dochází k podobným fyziologickým změnám během stárnutí jako u savců.

Introduction

Inflammation is a key immunological effector process that serves as a "double-edged sword" to fight infections on the one hand, but on the other hand can also cause tissue damage due to oxidative stress if it is misregulated (Graham, Allen and Read 2005; Ashley, Weil and Nelson 2012). For example, chronic self-propagating inflammation can contribute to the *inflammaging*, which is now widely regarded as one of the causes of senescence (Franceschi *et al.* 2007; Pawelec, Goldeck and Derhovanessian 2014; Pawelec 2018). Whether inflammation is triggered depends initially on the activation of specific innate immunity receptors on phagocytic cells, such as pattern recognition receptors (PRRs), which recognise specific pathogenic ligands as the lock-and-key system (Mogensen 2009; Kawai and Akira 2011). In natural populations, *Reed Queen dynamics* predicts great diversity in both the germ-encoded PRRs and their microbial ligands (Miller, Ernst and Bader 2005). While most microbial ligands originate from the commensal microbiota and normally only stimulate the immune system via PRRs to maintain intestinal homeostasis, some ligands originate from pathogens and can trigger a strong inflammatory response (Honda and Littman 2012; Eloe-Fadrosh and Rasko 2013; Ost and Round 2018). In the mammalian gut, for example, Firmicutes produce short-chain fatty acids (SCFAs) that generally prevent inflammation, while Bacteroidetes have more pro-inflammatory effects (Skillington *et al.* 2021). In young and healthy people, there is an equilibrium in the abundance of the major bacterial phyla, but this can be shifted towards an increased proportion of Bacteroidetes to Firmicutes in older people, leading to a pro-inflammatory state (Claesson *et al.* 2011; Skillington *et al.* 2021). Although these mechanisms are postulated in biomedical studies, these studies often work with laboratory models that have reduced variability in both immune genes and microbiota, and where natural selection is not taken into account or where individuals are kept under stable conditions (Pedersen and Babayan 2011). To better understand the evolution of inflammation, we need to go into the wild and study both interacting counterparts in natural populations. How much variation is there in the gut microbiota (GM) and innate immunity receptors that recognise microbial ligands in natural populations and how does it arise? Do *inflammaging* and physiological senescence follow similar trends in natural populations as in laboratory models? These are the fundamental questions that I, together with my colleagues, try to answer in this dissertation. Answering these questions is not only important to better understand the host-parasite arms race or the mechanisms of ageing, but uncovering these mechanisms could also have practical implications, e.g. for animal breeding or understanding resistance to infectious diseases (zoonosis). This is in line with the idea of the *One Health concept*, which assumes that human health is closely linked to animal health and the state of the entire ecosystem (Prata et al. 2022).

Firstly, I investigated GM variation within and between species and its evolutionary and ecological determinants in passerines. Despite the great diversity and ecological importance of birds (Oliveros *et al.* 2019), the study of GM in birds has lagged far behind that of mammals (Sun *et al.* 2022).

Furthermore, avian gastrointestinal tract (GIT) underwent multiple physiological and anatomical adaptations given active flight (Caviedes-Vidal *et al.* 2007; Mcwhorter, Caviedes-Vidal and Karasov 2009). The proportion of major bacterial phyla in birds also differs markedly from that of mammals (Hird *et al.* 2015; Grond *et al.* 2018). Wild bird GM can be driven by many extrinsic and intrinsic factors, but these are still relatively poorly understood compared to mammals (see recent reviews by Grond et al. 2018; Matheen et al. 2022; Sun et al. 2022). In our studies, we tested the effects of host phylogeny, ecological factors (such as diet or habitat), climatic conditions, seasonality and many other factors on microbial composition. Last, but not least, we tried to address the question of whether bird eggs are colonised with bacteria prior to hatching.

Secondly, I studied the intra- and inter-specific genetic diversity of innate immunity receptors (mainly PRRs) and the mechanisms that generate or maintain it. Compared to the widely studied adaptive immunity major compatibility genes (*MHC*; e.g. Kaufman 2022), the variability of innate immunity receptors is much less understood, and in particular Toll-like receptors (*TLRs*) and RIG-like receptors (*RLRs*), which directly interact with microbial ligands, could be promising candidates to explain variation in disease resistance (Kawai and Akira 2010; Rehwinkel and Gack 2020). As identifying adaptive (functional) variability of immune genes is challenging, we reviewed the current knowledge on vertebrate immune gene diversity as well as molecular methods and developed a novel methodological pipeline to predict functionally important single nucleotide variants (SNVs).

Thirdly, I explored physiological senescence and *inflammaging* in the great tit (*Parus major*), a model passerine bird for eco-evolutionary research. Small free-living passerines may be an ideal group for biomedical studies on longevity and senescence but are still neglected in biogerontological research. Although small birds have higher basal glucose and metabolic rate, they have high fertility even in old individuals and live about 2-3 years longer than mammals of similar size (Travin and Feniouk 2016). They also have some adaptations for longevity and coping with oxidative stress, such as relatively long telomeres, a higher proportion of unsaturated fatty acids in their membranes, higher antioxidant levels or proteins that prevent DNA damage and are more resistant to damage from glycosylation and glycoxidation (Holmes and Ottinger 2003; Travin and Feniouk 2016). Because birds produce fewer highly reactive compounds than mammals during acute inflammation (Genovese *et al.* 2013) we can assume that they suffer less from inflammaeging-related tissue damage, but virtually nothing is currently known about the role of *inflammaging* in avian senescence.

Research questions and aims

In my dissertation, I explored three interrelated topics: (1) ecological and evolutionary determinants of microbiota composition and diversity, a driver of wild bird immunity, (2) diversity in immune genes affecting inflammatory responses, and (3) inflammation-related physiological senescence in a passerine bird model, the great tit (*Parus major*). My general research questions for each topic and the specific objectives for each manuscript are as follows:

(1) What is the intra- and interspecific variation in the host gastrointestinal tract microbiota of wild birds? Which evolutionary and ecological factors drive microbial variation? How is the gastrointestinal tract microbiota formed during avian embryogenesis?

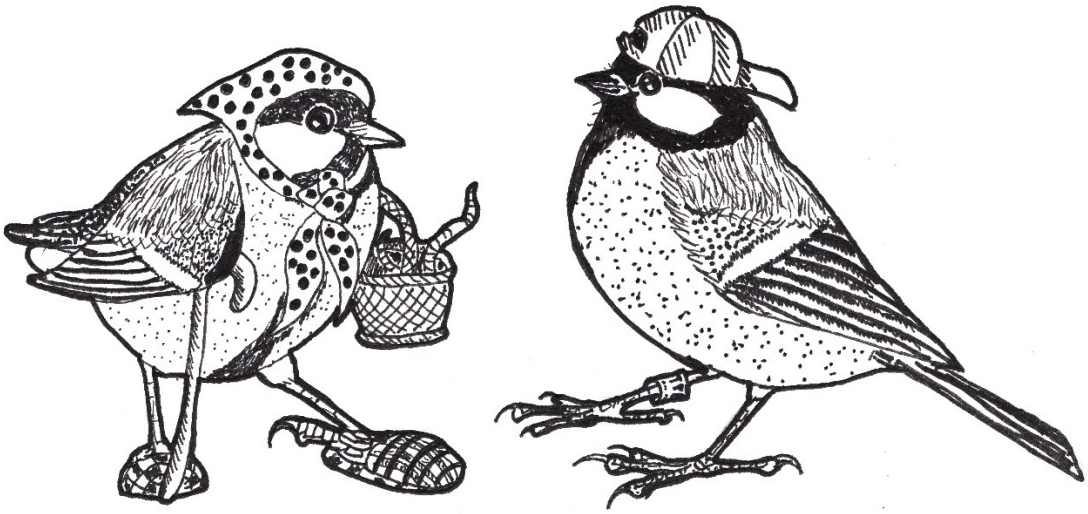
- To describe GM variation in passerines and ecological and evolutionary factors that determine it using large comparative interspecies passerine datasets (**papers I – III**)
- To describe variation of microbiota across different GIT tissues using selected passerine and parrot species (**papers IV – V**)
- To describe microbial communities in a bird egg shortly after laying and before the hatching in the great tit testing different mechanisms of bacterial colonisation (**paper VI**)

(2) How does genetic variation in innate immunity receptors between individuals and species contribute to differences in resistance to infectious diseases in wild birds? How does this variation evolve? Can we predict the adaptive functional effects of this variability using evolutionary bioinformatics tools?

- To review the current state of knowledge of immune gene diversity evolution in vertebrates and to propose new hypotheses to be tested (**paper VII**)
- To develop a methodological pipeline that identifies adaptive variation based on identifying adaptive convergence in any protein-coding genes (**paper VIII**)
- To describe overall and adaptive intra- and interspecific variation of selected *TLRs* and the evolutionary mechanisms that generate it in tits (**paper VIII**) and finches and buntings (**paper IX**)
- To describe evolution of *RLRs* (*RIG-I* and *MDA5*) in birds (**papers X – XI**)
- To describe evolution of cannabinoid receptors (*CNRs*) in birds and the functional consequences of *CNR2* gene loss (**paper XII**)

(3) Does senescence occur in various physiological and pro-inflammatory immune traits in a free-living passerine bird, the great tit? Do these traits follow the same, or different lifetime trajectories? What are the functional consequences of the observed age-related changes?

- To investigate whether plasma testosterone levels undergo age-related changes and whether testosterone levels affect various condition-related traits in the great tit (**paper XIII**)
- To test for age-related changes in inflammatory response and whether these are linked with changes in antioxidant/ oxidative stress marker levels in the great tit (**paper XIV**)
- To examine whether heavy metals accumulate in the blood over time and whether they have significant effects on individual health in the great tit **(paper XV**)



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Methods

Study species

In my dissertation, I used various bird species from different taxonomic levels, ranging from whole birds, through passerine and parrot clades, families of tits and finches and buntings to the great tit.

Microbial genotyping

For microbial studies, we collected samples either non-invasively from faeces, as these are a good proxy for gut communities (Videvall *et al.* 2018; Berlow, Kohl and Derryberry 2020), and oral swabs from wild mist-netted birds, or invasively from various tissue samples across a GIT gradient of euthanised captive individuals. Bacterial DNA was then isolated from different sample types stored deep-frozen in ethanol using the microbial extraction kit. Microbial communities were determined by NGS metabarcoding by amplifying the V3-V4 region of the *16S rRNA* gene using MiSeq Illumina paired-end sequencing. Where necessary, the abundance of selected amplicon sequence variants (ASVs) was further verified by a more accurate qPCR.

Assessing adaptive variation in immune genes

To describe the intra- and interspecific variation of immune genes in birds, we obtained sequences from different sources: (i) for genome-wide scans by downloading sequences from publicly available databases (ENSEMBL or NCBI), (ii) by targeted amplicon-based sequencing of individual ligand-binding region (LBR) of a gene (Illumina MiSeq and Sanger method) from genomic DNA extracted from blood and other tissues, and (iii) from the sequenced transcriptome of ileum tissue (mRNA-Seq by Illumina NextSeq 500). To detect adaptively evolving molecular convergence in immune genes, we developed a highly flexible pipeline that uses combinations of several advanced evolutionary bioinformatics approaches (such as positive selection and adaptive convergence testing combined with structural protein modelling). It consists of multiple filtering steps and decision points (schematically shown in Figure 1) that minimise the risk of false-positive single nucleotide variants (SNVs) and can be universally applied to all protein-coding genes.

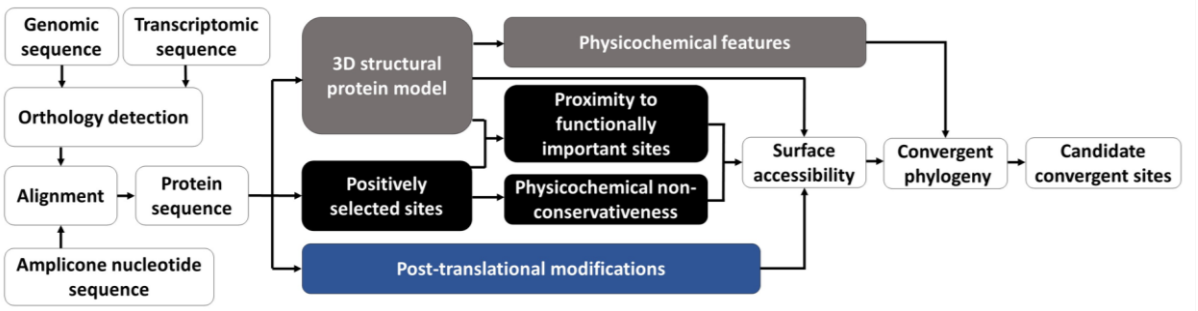

Figure 1: Scheme of the pipeline for identifying functionally relevant variation based on the detection of molecular convergence. Adapted from Těšický et al. (2020), wherein also see for more details.

Physiological senescence and health-related traits in the great tit

For all gerontological studies, we used a longitudinally monitored a free-living great tit population nesting in nest boxes in an urban forest on the outskirts of Prague (Ďáblický and Čimický háj, Czech Republic; 50°8'10.591 "N, 14°27'51.144 "E, ~ 315-360 m above sea level, total area ~ 0.9 km^2).

Various leucocyte and erythrocyte counts in the blood represent good proxies of health status and physiological stress in birds (Ots, Murumägi and Hõrak 1998; Davis, Maney and Maerz 2008). Using classical light microscopy in **haematological analysis**, we determined the relative white blood cell count (RWBC), immature erythrocyte count (IEC) and absolute white blood cell count (TWBC). For **background inflammation**, we measured the concentration of leukotriene B4 (LTB4; pro-inflammatory marker secreted by granulocytes) using high-performance liquid chromatography-electrospray ionisation-high-resolution mass spectrometry (HPLC-ESI1-HRMS) from fresh frozen blood. For **acute inflammation**, the oxidative burst of whole blood after *in vitro* lipopolysaccharide (LPS) stimulation was measured by the Pholasin-based chemiluminescence method using a commercially available kit. **Oxidative stress markers** were analysed from deep-frozen fresh blood using HPLC-ESI1-HRMS. We measured oxidation products of nucleic acids (8-hydroxy-2'-deoxyguanosine, 8-OHdG; 8-hydroxyguanosine, 8-OHG), proteins (o-tyrosine, O-Tyr; 3-nitrotyrosine, 3-NOTyr) and lipids (8-isoprostane, 8-ISO). For **antioxidant analysis**, we measured free reactive thiol levels as well the superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities separately from frozen whole blood based on colourimetric absorbance changes measured with a microplate reader. **Plasma testosterone** was measured with the testosterone ELISA kit from fresh frozen plasma obtained by centrifugation. **Heavy metal contamination of blood** with Pb, Cu, Zn and As was measured from whole blood samples stored in ethanol using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). To analyse **plumage ornamentation** (Quesada and Senar 2006; Hegyi et al. 2007), we assessed the size of melanin-breast stripe ornament from scanned individuals in a graphical programme and the spectral properties of the yellow breast plumage ornaments with a spectrophotometer. Both melanin and carotenoid-based plumage ornaments are condition-dependent traits that also serve as honest signals in sexual selection in birds (e.g. Albrecht et al. 2009; Svobodová et al. 2013; Guindre-Parker and Love 2014), including the great tit. **Feather growth rate** (FGR) of tail feathers was also assessed, as it is a condition-dependent trait that provides information on individual nutritional status during moult (Grubb 2006).

Results and discussion

Host microbiota in the gastrointestinal tract

Consistent with other studies in wild birds (Hird *et al.* 2015; Bodawatta *et al.* 2018; Grond *et al.* 2018, 2019), our results in adult GM confirmed the dominance of the major phyla Proteobacteria, Firmicutes, Actinobacteria, Chlamydiae and Bacteroidetes and high intra- and interspecific variation in avian GM (**paper I – V**), leaving most of the observed variability unexplained.

In **paper I**, we provide the first comprehensive insight into interspecific and interindividual GM variation of 53 old-world passerine species. Although previous mammalian studies have shown relatively strong effects of various life-history traits on GM profile, such as diet (Ley *et al.* 2008; Muegge *et al.* 2011; Gomez *et al.* 2019), we surprisingly found only very limited effects of host ecology (diet, habitat, etc.) and geography on GM composition. The strongest predictor of GM variation was host phylogeny. We propose that in GM evolution in passerines bacteria are first recruited from the environment and then selectively filtered by some host traits, such as immune genes, which could explain this co-diversification pattern.

In **paper II**, we directly compared for the first time the GM diversity and profile of 99 tropical and temperate passerine species (with residents/short-distance migrants and long-distance migrants) to identify potential GM drivers. Surprisingly, we identified no consistent differences in GM diversity and profile between tropical and temperate passerines. Interestingly, much more profound differences between dry and wet seasons were found in tropical passerine birds. To investigate the effects of migration on GM, we first directly compared the GM of two trans-Saharan migrants captured at both their wintering and breeding sites. Although one might expect substantial carry-over effects leading to mixing of their GM from both sites, GM of long-distance migrants converged well with the total GM of other passerines at the sampling site. This suggests that the overall GM is highly plastic, strongly influenced by the environment, and has limited OTU stability over time (see also Kreisinger et al. 2017; Risely et al. 2017, 2018 for further support). Second, when we compared GM profile between temperate trans-Saharan migrants and short-distance migrants or resident birds, GM did not differ in diversity and profiles, except for the abundance of lactic acid bacteria which was increased in long-distance migrants.

In following **paper III** when looking at the host GM-codivergence signal in more detail using the re-analysed dataset from **paper I**, we found that bacteria that interact closely with their host, e.g., affecting immune and metabolic functions showed stronger host specificity than other groups. Surprisingly, most other bacteria showed only little or no host specificity.

In **paper IV** we described high divergence of the great tit microbiota between two GIT sections, faecal samples representing GM and oral cavity. Diversity of the oral microbiota was much higher than in faeces, but with a relatively homogeneous composition, suggesting that the oral microbiota is mainly

recruited from the environment. In contrast, the less diverse faecal microbiota was much more inter-individually variable and did not correlate with the oral microbiota at the individual level. This may suggest that the faecal microbiota is more tightly regulated by some intrinsic mechanisms, such as the immune system. In the following **paper V**, we confirmed a clear divergence of microbial communities between the upper and lower GIT also in six parrot species. Moreover, continuous sampling in different lower GIT tissues revealed relatively homogeneous microbial communities between different sites, which corresponds well to the relatively low morphological diversity of the parrot gut. In this study, we also confirmed that faecal samples well represent gut microbiome (much better than cloacal swabs).

In paper VI, we came up with an innovative design to study the microbiome of bird eggs. In contrast to some previous studies in chickens (e.g. Ding et al. 2017, 2022; Lee et al. 2019; Akinyemi et al. 2020), which revealed a rather diversified microbiota in the egg contents and embryonic gut but largely neglected possible environmental contaminants, our results showed only negligible microbial communities in the great tit egg content and embryonic gut samples. We find that both *vertical bacterial transfer* from the oviduct and *bacterial trans-shell migration* could contribute to the formation of simple and low-abundant bacterial communities in bird egg before hatching, but their overall effects are very weak. This suggests that in passerines the main colonisation of the gut with bacteria occurs after hatching. Further studies on egg and embryonic microbiota across avian phylogeny are needed to determine whether the described discrepancy in microbiota recruitment between passerines and chickens reflects differences in life-history traits (e.g. *altricial* vs. *precocial* nestlings) or rather methodological differences between studies.

Evolution of innate immunity receptor diversity

Identifying functional immune gene variability involved in pathogen resistance or tolerance is a major challenge in evolutionary immunology (Vinkler, Adelman and Ardia 2022). The massive democratisation of transcriptomics and genomics platforms has opened up unprecedented opportunities for finding new immune gene candidates. But how do we find the needle in the haystack among thousands of immune genes?

In review **paper VII**, we first summarized the current state of knowledge about evolution of immune gene diversity in vertebrates and theoretically outlined the ways how to search for such candidate genes in animal genomes/ transcriptomes. Second, in **paper VIII** adopting the state-of-the-art predictive bioinformatics tools, we then practically outlined a new broadly applicable methodological approach for searching SNV candidates with high potential adaptive value based on identifying adaptive convergence. Our approach utilizes the identification of positively selected sites (PSS) or post-translational modifications in combination with 3D structural protein modelling, their various annotations, and the evaluation of whether they evolve in a convergent manner. By applying this

pipeline, we revealed that bacterial-sensing TLR4 and TLR5 LBRs in 29 tit species are well diversified at the interspecific level and subject to strong positive selection. The variability of tit *TLR* was of a similar order to other studies on bird and rodent *TLRs* (Alcaide and Edwards 2011; Fornůsková *et al.* 2013; Králová *et al.* 2018; Velová *et al.* 2018), but much lower than in *MHC* genes (Alcaide, Edwards and Negro 2007; O'Connor *et al.* 2016; Minias *et al.* 2021; Minias, He and Dunn 2021). Interestingly, we revealed that adaptive convergence was common in TLR4 and TLR5 LBRs and involved specific PSSs, post-translational modifications (N-glycosylation and phosphorylation) as well as the distribution of surface electrostatic charge that influence ligand binding.

Following this paper, as most *TLR* research in non-model bird species has focused on bottlenecked populations with depleted genetic variation (e.g. Grueber et al. 2013; Gonzalez-Quevedo et al. 2015; Vlček et al. 2022), in **paper IX**, we investigated intra- and interspecific variation in three *TLR* genes (*TLR1A*, *TLR3*, *TLR4*) in eleven species of buntings and finches with large effective population sizes. We find extraordinary TLR diversity, especially in *TLR1A* and *TLR4* genes and our evolutionary bioinformatics predictions further showed that most of this variation is putatively functional.

In **papers X** and **XI**, we found the widespread positive selection in *MDA5* and *RIG-I* in birds. However, using genome-wide *in silico* scans, we demonstrated that both *MDA5* (twice) and *RIG-I* (up to sixteen times) were lost in avian evolution, indicating the dynamic evolution of avian *RLRs*. This led us to hypothesise that the loss of one receptor might be compensated by the function of the other. Therefore, we tested whether adaptive compensatory evolution occurs in the molecular phenotype of *MDA5* in species where *RIG-I* had been pseudogenised and vice versa. While we did not detect specific compensatory adaptive evolution in *MDA5*-deficient species, we find some evidence for compensatory evolution in *MDA5* after *RIG-I* loss in Galliformes around dsRNA recognition sites. Future studies should clarify whether such frequent gene losses bring any advantages or whether species lacking one of these receptors are more susceptible to various viral infections, such as the highly pathogenic avian influenza (H5N1).

In **paper XII**, we also demonstrated gene loss of cannabinoid receptor 2 (*CNR2*), an important neuroimmune regulator, due to chromosomal re-arrangements in parrots. To test the functional effect of such pseudogenisation, we designed *in vitro* inflammation experiment and performed transcriptomic sequencing. Specifically, we experimentally induced peripheral sterile inflammation in six *CNR2*-deficient parrots and one passerine with intact *CNR2*, the zebra finch (*Taeniopygia guttata*). According to our predictions, we revealed that the experimentally induced peripheral inflammation propagated to the brain in the parrots but not in the zebra finch. All this suggests that *CNR2* gene loss might explain why parrots are more susceptible to neuroimmune diseases (Rinder *et al.* 2009; Staeheli, Rinder and Kaspers 2010) and infection-triggered behavioural disorders (Rubinstein and Lightfoot 2012; Speer 2014).

Physiological senescence

Since birds represent an analogous homeothermic lineage of amniotes with various adaptations for longevity, understanding molecular and physiological age-related changes may shed new light on the causes of senescence. Until recently, senescence in molecular physiological traits has been poorly documented in longitudinal studies of free-living birds (see reviews by Stier et al. 2015 and Bouwhuis and Vedder 2017) but see (e.g. Bize et al. 2014). Our studies on great tits are thus some of the first on wild passerine birds using a longitudinal dataset to reveal age-related trajectories in simultaneously measured multiple molecular and physiological traits.

In **paper XIII** we assessed inter-annual stability and age-related changes in plasma testosterone levels in 49 repeatedly captured great tits. In males, testosterone levels were only to a limited extent interannually repeatable and had polynomic dependence on age, peaking in mid-life and followed by a steady decline. Such a trajectory in which testosterone levels co-vary with the drop in reproductive performance (Ottinger 1996) was expected in birds but previous non-longitudinal studies have brought much controversy in testosterone lifetime dynamics (e.g. Peters et al. 2002; Smith et al. 2005; Madsen et al. 2007). Our study thus provides evidence for *endocrinological senescence* in male testosterone levels as in mammals. As testosterone regulates multiple condition-related traits, including ornamental expression (Duckworth, Mendonça and Hill 2004; Galván, Díaz and Sanz 2010; Vinkler and Albrecht 2010) and generally has an immunosuppressive effect (Foo *et al.* 2017), we also tested for its association with sexually selected yellow breast ornamentation and black melanin stripe (Senar and Quesada 2006; Hegyi *et al.* 2007), heterophil: lymphocyte ratio indicating physiological stress (Davis et al. 2008) and FGR (Grubb 2006). The only yellow brightness in males was positively correlated with plasma testosterone levels. This is an interesting finding, as this ornament serves as a quality indicator in tits (Senar, Figuerola and Pascual 2002) and may play a role in sexual selection.

In **paper XIV** we compared lifetime patterns of different pro-inflammatory traits: (i) chronic inflammation measured as leukotriene B4 levels (LTB4) and (ii) acute inflammation measured as cellular oxidative burst after *in vitro* LPS challenge in 54 repeatedly captured great tits. LTB4 is a pro-inflammatory lipid regulator that is elevated in various chronic inflammatory age-related diseases in humans (He et al. 2020). As we found that LTB4 levels also increased linearly with age in great tits, this brings the first evidence that birds can suffer from *inflammaeging* as humans (Franceschi *et al.* 2000, 2007). In contrast, the cellular oxidative burst response showed a polynomial dependence on age, with the highest peak in midlife followed by a steady decline. This clearly illustrates the functional *immunosenecence* in general effector cellular inflammatory response. We found negative correlation between GPX activity and LTB4 levels, suggesting that chronic inflammation depletes the antioxidant availability for free-radical clearance, which may induce further oxidative stress and promote further inflammation (Pisoschi and Pop 2015). Interestingly, in our unpublished results using the same longitudinal dataset, we found that oxidative damage accumulated progressively with age and these

observed trends were highly consistent across all nucleic acid, protein and lipid markers (Těšický et al., *in prep*.).

In **paper XV** we evaluated longitudinal accumulation of four heavy metals (Zn, Pb, Cd, As) in blood and their possible effects on health-related haematological traits in 185 recaptured great tits. Contrary to our predictions, we found weak age-related changes in blood only for Pb with the highest levels occurring in nestlings and the oldest senescent individuals and not in other heavy metals. In contrast to previous studies (Bauerová et al. 2017), no associations existed between heavy metals and anaemia-like conditions. This is probably due to sampling in relatively low-pollution habitats. Nevertheless, we found that TWBC were positively correlated with all three heavy metal levels assessed, suggesting that their toxicity may increase leucocyte proliferation (Dumonceaux and Harrison 1994; Jones 2015). Our results demonstrate that bird blood can be used for actual heavy metal monitoring, even if the age is not known.

Conclusion

In my work, I have provided some novel insights into the evolution of inflammation and antimicrobial resistance in birds. By studying (1) ecological and evolutionary determinants of GM composition and diversity that activate and modulate inflammation, (2) diversity of immune genes that influence inflammatory responses, and (3) inflammation-induced physiological senescence in wild birds, I have contributed to expanding our knowledge of host-pathogen co-evolution in birds, paving the way for future research in microbial ecology, evolutionary immunology and biogerontology (not only) in birds.

We found high intra- and interspecific variation in passerine GM (**papers I – IV**), but this remained largely unexplained in our studies. Host phylogeny and, to a very limited extent, also host ecology shaped interspecific variation of GM in passerines, yet their overall contribution was much smaller than in similar studies in mammals. Our studies also showed that GM is significantly influenced by environmental conditions and is highly plastic. The lower GIT showed relatively homogenous microbial composition at different sites (**paper V**), which is consistent with the relatively homogeneous gut morphology in flying birds, probably as an adaptation to active flight (Caviedes-Vidal *et al.* 2007). It has been hypothesised that the high unexplained variability and limited stability of operational taxonomic units (OTUs) in passerine GM may be because a large proportion of GM only rapidly passes through the passerine gut and does not colonise it (Schmiedová 2022). Aiming to further clarify how and when the first bacteria colonise the bird egg, we revealed that bird egg is nearly sterile in the great tit (**paper VI**). This suggests that in passerines GM is predominantly formed after hatching. All our results thus document that bird GM differs significantly from the GM of mammals and that the results of studies on mammals cannot generally be applied to birds. In the future, microbial studies in birds should focus more on the understanding functional role of GM and its interactions with the immune system.

The conceptual framework developed for categorising immune genes (**paper VII**), together with our newly outlined *state-of-the-art* methodological pipeline identifying putative functional variation based on adaptive convergence (**paper VIII**), can be applied to systematically search for functional SNVs in antiparasitic resistance in immune genes across genomes far beyond the best-studied *MHC*. This can also have multiple practical implications, e.g. in animal breeding, in predicting the zoonotic potential of infectious diseases, or in conservation genetics, where decisions about which populations to prioritise conservationally can be better made on assessing adaptive variability encoded in immune genes rather than by neutral markers alone. Indeed, our screening of innate immunity TLRs (**papers VIII – IX**) and RLRs (**papers X – XI**) in passerines revealed that they are highly variable, subject to positive selection and that many SNVs appear to be functionally important for pathogen recognition but are still waiting to be functionally verified by *in vitro* mutagenesis. Striking multiple gene losses of *MDA5* and *RIG-I* in different avian clades opened the way for a functional understanding of such an event. Our reported pseudogenisation of *CNR2* in parrots (**paper XII**) could explain why parrots are more susceptible to various inflammation-triggered psychological and neuroimmune disorders. And more importantly, given the advanced cognitive abilities of parrots, this might suggest that parrots in captivity could serve as a promising biomedical model for studying various inflammation-related behavioural syndromes.

Using a longitudinally monitored population of the free-living great tit, we have provided robust evidence for physiological senescence and *immunosenescence* in multiple traits (**papers XIII – XV** and Tesicky et. al., *in prep.*). However, the lifetime trajectories of the various markers differed. During ageing, individuals linearly accumulated various oxidative tissue damage to nucleic acids, proteins and lipids. Chronic inflammation was also linearly elevated, demonstrating for the first time *inflammaging* in birds. In contrast, male testosterone and cellular oxidative burst levels were polynomially dependent on age, corresponding with *hormonal senescence* and *immunosenescence*. All this shows that small passerine birds appear to age as humans and laboratory mammals, despite multiple adaptations for longevity. Future studies should reveal the costs of such trajectories, e.g. through the effects on fitness. While the observed senescence in some traits may be a passive consequence of physiological decline without functional constraints, its decline in other traits may be driven by excessive costs to maintain them, followed by active diversion from energetically costly traits (Letters 2018; Gaillard and Lemaître 2020).

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Vinkler M, Adelman JS, Ardia DR. Chapter 20 - Evolutionary and ecological immunology. In: Kaspers B, Schat KA, Göbel TW, et al. (eds.). Boston: Academic Press, 2022, 519–57.

Vinkler M, Albrecht T. Carotenoid maintenance handicap and the physiology of carotenoid-based signalisation of health. *Naturwissenschaften* 2010;**97**:19–28.

Vlček J, Miláček M, Vinkler M *et al.* Effect of population size and selection on Toll-like receptor diversity in populations of Galápagos mockingbirds. *J Evol Biol* 2022, DOI: 10.1111/jeb.14121.

Curriculum Vitae

Name: Martin Těšický

Year of birth: 1991

Place of birth: Valašské Meziříčí, Czech Republic

e-mail: martin.tesicky@natur.cuni.cz

Research identifier: ResearcherID: L-3370-2017, ORCID: 0000-0001-8097-5331,
https://www.researchgate.net/profile/Martin_Tesicky,
https://scholar.google.cz/citations?user=u0HDtVEAAAAJ&hl=cs

EDUCATION

2016 – Doctoral studies – **Zoology**, Department of Zoology, Charles University, Prague**,** dissertation thesis *Host-microbiota, pro-inflammatory immunity and physiological senescence in wild birds* (Supervisor: Dr. Michal Vinkler)

2014 – 2016 Master studies – **Zoology**, Department of Zoology, Charles University, Prague, master thesis: *Trans-species polymorphism in selected genes of innate immunity in tits (Paridae)* (Supervisor: Dr. Michal Vinkler), awarded by dean´s award for the best master thesis

2011 – 2014 Bachelor studies – **Biology**, Charles University, Prague, bachelor thesis: *Trans-species polymorphism in immune genes at wildlife animals* (Supervisor: Dr. Michal Vinkler)

2005 – 2011 Grammar School of František Palacký Valašské Meziříčí

PROFESSIONAL CAREER

2017 – Research assistant, Department of Zoology, Faculty of Science, Charles University, Prague

2015 – 2016 Technical assistant, Department of Zoology, Faculty of Science, Charles University, Prague

PROFESSIONAL INTERESTS

Evolutionary genetics, immunogenetics, evolutionary and ecological immunology, evolutionary biology, ornithology

RESEARCH EXPERIENCE

Participation in 11 research projects: as a principal investigator (1), a co-investigator (4) or as a member of the research team (6)

2023 – Participation in a grant project of The Czech Science Foundation, grant No. 23-07216S: *Interplay between genetics, ecology and symbionts in two adaptive radiations of New Guinean rodents* (PI Jakub Kreisinger)

2021 – **Co-investigator** in a student grant project of Charles University, grant No. START/SCI/113: The avian inflammasome: gene evolution, expression, and interactions with microbiota following immunological challenges (PI Baleraj Melepat)

2018 – 2022 Participation in a grant project of The Czech Science Foundation, grant No. P502/19-20152Y: *Effects of microbiota composition on inflammation immunity and behavioural syndroms in birds* (PI Michal Vinkler)

2018 – 2020 Participation in a grant project of Charles University, grant No. PRIMUS/17/SCI/12: *Impact of neuroinflammation on brain development and learning in cognitively advanced birds* (PI Michal Vinkler)

2018 – 2021 **Co-investigator** in a grant project of the Charles University Grant Agency, grant No. 1626218: *Geographic variability of heavy metal contamination and its effects on health and aging in the great tit (Parus major)* (PI Tereza Krajzingrová)

2018 – 2019 **Co-investigator** in a grant project of the Ministry of Education, Youth and Sports, grant No. InterCost LTC18060: *Mapping of diversity in avian genomic resources available in the Czech Republic* (PI Michal Vinkler)

2017 – 2019 **Principal investigator** in a grant project of the Charles University Grant Agency, grant No. 1158217: *Microbiome of bird egg in early and late embryogenesis: impact of experimental* in ovo *probiotics administration on establishment of microbiota and gene expression of immune genes in birds* (PI Martin Těšický)

2015 – 2017 Participation in a grant project of The Czech Science Foundation, grant No. 15-11782S: *Biology of ageing: mechanisms and patterns of senescence in free-living birds* (PI Tomáš Albrecht)

2014 – 2016 **Co-participant** in a grant project of the Charles University Grant Agency, grant No. 540214: *Trans-species polymorphism in selected genes of innate and acquired immunity in tits (Paridae)* (PI Hana Velová)

2014 – 2016 Participation in a grant project of The Czech Science Foundation, grant No. 14-16596P: *Interaction between ecological traits in birds and their gastrointestinal microbiota: Metagenomic approach* (PI Jakub Kreisinger)

2012 – 2014 Participation in a grant project of The Czech Science Foundation, grant No. P505/10/1871: *Toll-like receptors in passerine birds: description, characterization of polymorphism and evolutionary consequences of allelic* variation (PI Josef Bryja)

RESEARCH SKILLS

- Extensive ornithological field experience
- Extensive knowledge of molecular-genetic and laboratory methods (DNA purification, PCR, qPCR, gel electrophoresis, Sanger and MiSeq sequencing)
- Structural bioinformatics (protein 3D modelling, surface charge analysis)
- Computational bioinformatics (amplicon Illumina MiSeq data analysis, transcriptome analysis of Oxford Nanopore and NextSeq data, sequence and positive selection analyses)
- Linux (bash), R-programming
- Statistics (GLM and GLMM models)

MOBILITY

2021 Research visit at Jagiellonian University, Faculty of Biology, Institute of Earth Sciences, Genomics and Experimental Evolution Group by prof. Wiesław Babik (internship focused on screening for positive selection in avian immune genes from bird genomes) (9 months; ERASMUS and START)

2019 – 2020 Research visit at Jagiellonian University, Faculty of Biology, Institute of Earth Sciences, Genomics and Experimental Evolution Group by prof. Wiesław Babik (internship focused on the processing of Oxford Nanopore RNA-seq data) (7 months; ERASMUS)

2015 Study stay at the University of Oslo, The Faculty of Mathematics and Natural Sciences, Centre for Ecological and Evolutionary Synthesis, House Sparrow Group by prof. Glenn-Peter Sætre (5 months; ERASMUS)

COURSES ATTENDED

21.06. – 16.7. 2021	Practical Computing for Biologists workshop (Jagiellonian University, Krakow, Poland)
24. – 28.06. 2019	Ph.D. and postdoc training school on ChIP-seq (wet-lab) and basic functional animal genome analysis, Wageningen, Netherlands
3. – 5.5. 2019	MICOBION workshop on omics data integration & advanced course on metagenomics and omics data, BIOCEV, Prague
2015 –	Licence for animal manipulations in scientific experiments
2013 –	Bird ringing course – bird ringing license for the Czech Republic
3. – 7.9. 2012	Summer School of Molecular Biology – Mohelno, Mohelno, Czech Republic
23. – 30.6. 2012	23rd International Wildlife Research Week 2012, Swiss Youth in Science (SJF), Valchava, Switzerland

PUBLICATION ACTIVITY

Number of publications: 13 articles in IF-journals (currently at WOS 9; valid 16/01/2023)
Number of citations: 111/172 (WOS/ Google Scholar)
Number of citations without auto-citations: 105 (WOS)
Citing Articles without self-citations: 105
Average number of citations per article: 12.33
H-index: 4/5 (WOS/ Google Scholar)

Published papers

13. Schmiedová, L., Kreisinger, J., Kubovčiak, J., **Těšický, M.**, Martin, F.-P.J., Tomášek, O., Kauzálová, T., Sedláček, O. & Albrecht, T. (2023). Gut microbiota variation between climatic zones and due to migration strategy in passerine bird. *Frontiers in Microbiology*, 1–13. doi.org/10.3389/fmicb.2023.1080017 [IF_{2022} = 6.06]

12. Krchlíková, V., Hron, T., **Těšický, M.**, Li, T., Ungrová, L., Hejnar, J., Vinkler, M. & Elleder, D. (2023). Dynamic evolution of avian RNA virus sensors: Repeated loss of RIG-I and RIPLET. *Viruses, (1)*15, 1-15. doi.org/10.3390/v15010003 [IF_{2022} = 5.81]

11. Divín, D., Goméz Samblas, M., Kuttiyarthu Veetil, N., Voukali, E., Świderská, Z., Krajzingrová, T., **Těšický, M.**, Beneš, V., Elleder, D., Bartoš, O. & Vinkler, M. (2022): Cannabinoid receptor loss makes parrots susceptible to neuroinflammation. *Proceedings of the Royal Society B*, 289. doi: 10.1098/rspb.2022.1941 [IF_{2022}= 5.53]

10. Kubovčiak, J., Schmiedová, L., Albrecht, T., **Těšický, M.**, Tomášek, O., Kauzálová, T. & Kreisinger, J. (2022). Within-community variation of interspecific divergence patterns in passerine gut microbiota. *Ecology and Evolution*, 12:e9071. https://doi.org/10.1002/ece3.9071 [IF_{2022} = 2.77]

9. **Těšický, M.**, Krajzingrová, T., Eliáš, J., Velová, H., Svobodová, J., Bauerová, P., … Vinkler, M. (2022). Inter-annual repeatability and age-dependent changes in plasma testosterone levels in a longitudinally monitored free-living passerine bird. *Oecologia*, *198*(1), 53–66. doi: 10.1007/s00442-021-05077-5 [IF_{2022} = 3.23]

8. Krchlíková, V., Hron, T., **Těšický, M**., Li, T., Hejnar, J., Vinkler, M., & Elleder, D. (2021). Repeated MDA5 gene loss in birds: An evolutionary perspective. *Viruses*, *13*(11), 1–12. doi: 10.3390/v13112131 [IF_{2021} = 5.81]

7. **Těšický, M.**, Krajzingrová, T., Świderská, Z., Syslová, K., Bílková, B., Eliáš, J., … & Vinkler, M. (2021). Longitudinal evidence for immunosenescence and inflammaging in free-living great tits. *Experimental Gerontology*, *154*, 111527. doi: https://doi.org/10.1016/j.exger.2021.111527 [IF_{2021} = 4.03]

6. Palomar G., Dudek K., Wielstra, B., Jockusch, E. L., Vinkler, M., Arntzen, J.W., Ficetola, G.F., Matsunami, M., Waldman, B., **Těšický, M.**, Zieliński, P. & Babik, W. (2021). Molecular Evolution of Antigen-Processing Genes in Salamanders: Do They Coevolve with MHC Class I Genes? *Genome Biology and Evolution, 13:1–15. https://doi.org/10.1093/gbe/evaa259* [IF_{2021} = 3.42]

5. **Těšický, M.**, Velová, H., Novotný, M., Kreisinger, J., Beneš, V. & Vinkler, M., 2020. Positive selection and convergent evolution shape molecular phenotypic traits of innate immunity receptors in tits (Paridae). *Molecular Ecology*, (April), 3056–3070. doi: 10.1111/mec.15547 [IF_{2020} = 6.91]

4. Bauerová, P., Krajzingrová, T., **Těšický, M.**, Velová, H., Hraníček, J., Musil, S., Svobodová,J., Albrecht, T. & Vinkler, M. (2020). Longitudinally monitored lifetime changes in blood heavy metal concentrations and their health effects in urban birds. *Science of the Total Environment*, *723*. doi: 10.1016/j.scitotenv.2020.138002 [IF_{2020} = 7.96]

3. Kropáčková, L., Pechmanová, H., Vinkler, M., Svobodová, J., Velová, H., **Těšický, M**., Martin, F. P.J. & Kreisinger, J. (2017) Variation between the oral and faecal microbiota in a free-living passerine bird, the great tit (*Parus major*). *PLoS ONE*, *12*(6). doi: 10.1371/journal.pone.0179945 [IF_{2017} = 2.81]

2. Kropáčková, L., **Těšický, M.**, Albrecht, T., Kubovčiak, J., Čížková, D., Tomášek, O., Martin, F.-P.J., Bobek, L., Králová, T., Procházka, P. & Kreisinger, J. (2017) Co-diversification of gastrointestinal microbiota and phylogeny in passerines is not explained by ecological divergence. *Molecular Ecology*, *26*(19), 5292–5304. doi: 10.1111/mec.14144 [IF_{2017} = 6.09]

1. **Těšický M.** & Vinkler, M. (2015) Trans-Species Polymorphism in Immune Genes: General Pattern or MHC-Restricted Phenomenon? *Journal of Immunology Research*, 10:838035. [IF_{2015} = 2.92]

Submitted:

1. **Těšický, M.**, Schmiedová, L., Krajzingrová, T., Gómez Samblás, M.M., Bauerová, P., Kreisinger, J. & Vinkler, M.: Nearly (?) sterile avian egg in passerine bird (*submitted to FEMS Microbiology Ecology*)

2. Veetil, N.K., Oliveira, H.C., Gómez Samblás, M.M., Divín, D., Melepat, B., Voukali, E., Świderska, Z., Krajzingrová, T., **Těšický, M.**, Beneš, V., Madsen, O. & Vinkler, M. Application of the 3' mRNA transcriptomic sequencing (QuantSeq) for identification of differential gene expression during neuroinflammation in the zebra finch (*submitted to Immune Network*)

3. Schmiedová, L., Černá, K., Li, T., **Těšický, M.**, Kreisinger, J. & Vinkler, M.: Bacterial communities along parrot digestive and respiratory tracts: the effects of sample type, species and time *(submitted to International Microbiology*)

4. Vinkler, M., Fiddaman, S.R., **Těšický, M.**, O'Connor, E.A., Savage A.E, Lenz, T.L., Smith, A.L., Kaufman, J., Bolnick, D., Davies, CH.L., Dedic, N., Flies, A.S., Gómez Samblás, M. M., Henschen, A., Novák, K., Palomar, G., Raven, N., Samake, K., Slade, J., Veetil, N. K., Voukali, E., Höglund, J., Richardson, D.S. & Westerdahl, H.: Understanding the evolution of immune genes in vertebrates (*submitted to Journal of Evolutionary Biology*)

5. Włodarczyk, R., **Těšický, M.**, Vinkler M., Novotný, M., Remisiewicz, M., Janiszewski, T. & Minias, P.: Divergent evolution drives high toll-like receptor (TLR) diversity in passerine birds: buntings and finches (*submitted to Developmental and Comparative Immunology*)

CONFERENCES

17 talks and 24 poster presentations at international and national conferences (including Evolutionary Biology Meeting at Marseille 2016, ESEB 2017, 2019, 2021, 2022, International ornithological congress 2018, etc.; see for a complete list below)

TEACHING ACTIVITIES

- Teaching in practical courses at the Department of Zoology, Faculty of Science, Charles University, Prague (since 2017): Vertebrate Zoology (MB170P13A), Morphology of animals (MB170C46A), Field Course in Zoology (MB170T24)
- Co-organizing Evolutionary Genomics Journal Club (open discussion seminar, twice per month) (2017-2018)
- Student supervision: Dominika Soukupová (thesis: Microbiome of avian egg in the great tit (*Parus major*), participation in the national round of Students´ Professional Activities competition in 2020)

MEMBERSHIP IN SOCIETY AND ORGANIZATION

2022 –	Czech Immunological Society
2018 –	International Ornithologist's Union
2017 –	European Society for Evolutionary Biology
2013 –	Společnost spolupracovníků Kroužkovací stanice Národního muzea Praha (The Society of co-workers in Bird Ringing Center, National Museum in Prague)
2009 –	Czech Society for Ornithology
2018 – 2019	Student member of The Academic Senate of the Faculty of Science of Charles University
2014 – 2019	A member of the working group for Biological Olympiad

AWARDS

2020 Junior Thylacine award for best student paper at Department of Zoology, Faculty of Science, Charles University

2018 Queen Jadwiga Fund Scholarship, Jagiellonian University, Krakow

2016 Dean´s award for best master thesis at Faculty of Science, Charles University

2011 1st place in the national round of Students´ Professional Activities competition (2011) (Středoškolská odborná činnost) for the thesis: *The comparison of semiquantitative ornithological methods used in a middle-sized area*

COMMUNITY SERVICES

- Paper reviews: Genome Biology and Evolution (3), Infection, Genetics and Evolution (1), Plose ONE (1)
- Participation in organizing of conference: 2nd FAANG-Europe conference 2020, Prague

SCIENCE POPULARIZATION

- Lecturer of ornithology and biology for talented high school and primary school students: Biologická olympiáda, Arachne, Flurescenční noc, Zelená stezka-Zlatý list etc.
- Bird excursion for the public within Czech Society for Ornithology (Dawn chorus day, etc.)

OTHER SKILLS

Language skills: English (B2), German (B1)

Driving licence: B

LIST OF CONFERENCE CONTRIBUTIONS

41. **Těšický, M.**, Babik, W., Bartoš, O., Beneš, V., Vinkler, M.: Different immune genes show variation in strength of positive selection: genomic approach in birds. The 2022 Congress of the European Society for Evolutionary Biology, Prague, Czech Republic 2022 (poster; English)

40. Li, T., **Těšický, M.**, Melepat, B., Divín, D., Veetil, N.K., Vinkler, M.: Adaptive evolution of inflammasome-related genes in amniotic vertebrates. The 2022 Congress of the European Society for Evolutionary Biology, Prague, Czech Republic 2022 (poster; English)

39. Vinkler, M., **Těšický, M.**, Krajzingrová, T., Świderská, Z., Syslová, K, Barbora Bílková, Jiří Eliáš, Hana Velová, Petra Bauerová, Tomáš Albrecht & Jana Svobodová: Biomarkers for immunosenescence and inflammaging in wild passerine birds. The 2022 Congress of the European Society for Evolutionary Biology, Prague, Czech Republic 2022 (poster; English)

38. Gomez Samblas, M.M., Divín, D., Veetil, N.K., Melepat, M., **Těšický, M.**, Vinkler, M.: Variation in immune responses on intraspecific and interspecific levels in parrots. The 2022 Congress of the European Society for Evolutionary Biology, Prague, Czech Republic 2022 (poster; English)

37. Schmiedová, L., Krajzingrová, T., Bauerová, P., **Těšický, M.**, Pinkasová, H., Divín, D., Musil, S., Hraníček, J., Kreisinger, J., Vinkler, M.: The drivers of gut microbiota in wild great tit: effects of health and heavy metal contamination. The 2022 Congress of the European Society for Evolutionary Biology, Prague, Czech Republic 2022 (poster; English)

36. Divín, D., Goméz Samblas, M.M., Veetil, N.K., Voukali, E., Świderska, Z., Krajzingrová, T., **Těšický, M.**, Elleder, D., Bartoš, O.: Chromosomal rearrangements are responsible for altered neuroinflammatory regulation in parrots. The 2022 Congress of the European Society for Evolutionary Biology, Prague, Czech Republic 2022 (poster; English)

35. Divín, D., Goméz Samblas, M., Kuttiyarthu Veetil, N., Voukali, E., Swiderska, Z., Krajzingrová, K., **Těšický, M.**, Beneš, M., Elleder, D., Vinkler, M.: Inflammation regulator gene loss in parrots. ESEB Satelitte symposium: 7. Molecular evolution of the vertebrate immune system, from the lab to natural populations, Prague, Czech Republic 2021 [online] (lecture; English)

34. **Těšický, M.**, Velová, H., Novotný, M., Kreisinger, J., Beneš, V., Vinkler, M.: Detection of adaptive convergence in immune genes: a lesson from Toll-like receptors in tits. ESEB Satelitte symposium: 7. Molecular evolution of the vertebrate immune system, from the lab to natural populations, Prague, Czech Republic 2021 [online] (lecture; English)

33. **Těšický, M.**, Babik, W., Zielinski, P., Veetel Kuttiyarthu, N. Gomez Samblas M., Beneš, V., Vinkler, M.: Processing of long-read transcriptomic data for evaluation of positive selection acting on immune genes in parrots. The Fourth Eco Evo Ph.D. meeting, Lutherstadt, Wittenberg, Germany 2020 (poster; English)

32. **Těšický, M.**, Velová, H., Novotný, M., Kreisinger, M., Beneš, V. a Vinkler, M.: Positive selection and convergent evolution shape molecular phenotypic traits of innate immunity receptors in tits

(Paridae). The Fourth Eco Evo Ph.D. meeting, Lutherstadt, Wittenberg, Germany 2020 (lecture; English)

31. **Těšický, M,** Krajzingrová, T, Velová, H, Svobodová, J, Bauerová, P, Albrecht, T, Vinkler, M: Age-dependent changes in plasma testosterone level in a longitudinally monitored free-living population of the great tit (Parus major), The Zoological days, Olomouc, Czech Republic 2020 (lecture; Czech)

30. Bauerová, P., Krajzingrová, T., **Těšický, M.**, Velová, H., Hraníček, J., Musil, S., Svobodová, J., Albrecht, T., Vinkler, M.: Bioakumulace těžkých kovů v krvi a její projevy na parametrech zdravotního stavu u dlouhodobě sledované městské populace sýkor koňader (*Parus major*), The Zoological days, Olomouc, Czech Republic 2020 (lecture; Czech)

29. **Těšický, M.**, Tomášek, O., Krajzingrová, T., Velová, H., Svobodová, J., Bauerová, P., Pinkasová, H., Albrecht, T., Vinkler, M.: Effect of ageing on immunity, antioxidant enzymes and markers of oxidative stress in great tits (*Parus major*). The 3rd Evolutionary and ecological Ph.D. meeting, Bayrueth, Germany, 2019 (lecture; English)

28. **Těšický, M.**, Tomášek, O., Syslová, K., Krajzingrová, T., Hraníček, J., Velová, H., Svobodová, J., Bauerová, P., Pinkasová, H., Albrecht, T., Vinkler, M.: Ageing in free-living great tits: multimarker evidence for age-related increase in oxidative and physiological stress. The 2019 Congress of the European Society for Evolutionary Biology, Turku, Finland 2019 (lecture; English)

27. Vinkler, M., Velová, H., Świderská, Z., Voogdt, C. G. P., **Těšický, M.**, Putten J., Beneš, V.: Colour encoded in innate immune gene? Accumulating evidence for Hamilton-Zuk 'Good genes' in great tits. The 2019 Congress of the European Society for Evolutionary Biology, Turku, Finland 2019 (lecture; English)

26. **Těšický M.**, Tomášek, O., Syslová, K., Krajzingrová, T., Velová, H., Svobodová, J., Bauerová, P., Pinkasová, H., Albrecht, T., Vinkler, M.: The physiological aspects of ageing in free-living population of great tits (*Parus major*). The Zoological days, Brno, Czech Republic 2019 (lecture; Czech)

25. **Těšický, M.**, Velová, H., Novotný, M., Kreisinger, J., Vinkler, M.: Positive selection and convergent evolution shape molecular phenotypic traits of innate immunity receptors in tits (Paridae). The Zoological days, Prague, Czech Republic 2019 (poster; English)

24. Krajzingrová, T., Bauerová, P., **Těšický, M.**, Velová, H., Svobodová, J., Musil, S., Albrecht, T., Hraníček J., Vinkler M.: Pollution and age-dependent changes in haematological traits in great

tits and their association with reproduction. The Zoological days, Brno, Czech Republic 2019 (poster; English)

23. **Těšický, M.**, Velová, H., Novotný, M., Kreisinger, J., Vinkler, M.: Positive selection and convergent evolution shape molecular phenotypic traits of innate immunity receptors in tits (Paridae). The Zoological days, Prague, Czech Republic 2019 (poster; English)

22. Kubovčiak, J., Kropáčková, L., Albrecht, T., **Těšický, M.**, Martin, J.F., Kreisinger, J.: Analysis of holobiont associations between host and intestinal microbiota in passerines. The Zoological days, Brno, Czech Republic 2019 (poster; English)

21. **Těšický, M.**, Velová, H., Novotný, M., Kreisinger, J., Vinkler, M.: Positive selection and convergent evolution shape molecular phenotypic traits of innate immunity receptors in tits (Paridae). International ornithological congress, Vancouver, Canada, 2018 (poster; English).

20. **Těšický, M.**, Velová, H., Svobodová, J., Bauerová, P., Krajzingrová, T., Tomášek, O., Pechmanová, H., Albrecht, T., Vinkler, M.: The older, the better? How ageing affects plumage coloration, reproductive investment and immunity in great tits (*Parus major*). PhD meeting in animal evolutionary biology (Berlin – Dresden – Prague – Bayreuth), Svatý Jan pod Skalou, Czech Republic 2018 (lecture; English)

19. **Těšický, M.**, Velová, H., Svobodová, J., Bauerová, P., Krajzingrová, T., Tomášek, O., Pechmanová, H., Albrecht, T., Vinkler, M.: Biology of ageing and immunosenescence in great tit (*Parus major*). The Zoological days, Prague, Czech Republic 2018 (lecture; Czech)

18. Krajzingrová, T., **Těšický, M.**, Velová, H., Svobodová, J., Bauerová, P., Albrecht, T., Vinkler, M.: Vliv věku na hematologické znaky u sýkory koňadry (*Parus major*). The effect of age on hematological traits in great tits *(Parus major).* The Zoological days, Prague, Czech Republic 2018 (poster; Czech)

17. Kubovčiak, J., Kropáčková, L., Albrecht, T., **Těšický, M.**, Martin, J.F., Kreisinger, J.: Analysis of predictions of holobiont concept in microbiota-host relationship in passerines. The Zoological days, Prague, Czech Republic 2018 (poster; Czech)

16. **Těšický, M.**, Krajzingrová, T., Świederská, Z., Velová, H., Kreisinger, J., Vinkler, M.: When do bacteria colonize bird egg? Microbiome of avian egg in early and late embryogenesis and its impact on gene expression of immunne genes in birds. The Zoological days, Prague, Czech Republic 2018 (poster; Czech)

15. **Těšický, M.**, Velová, H., Reifová, R., Vinkler, M.: Different species yet the same alleles: shared polymorphism in innate immunity receptors in the tit family. Congress of the European Society for Evolutionary Biology (ESEB), Groningen, Netherlands 2017 (lecture; English)

14. **Těšický, M.**, Velová, H., Reifová, R., Vinkler, M.: Different species yet the same alleles: shared polymorphism in innate immunity receptors in the tit family. Conflict and Cooperation – Bridging Evolution, Ecology and Immunology, Ph.D. Student Meeting, Bautzen, Germany 2017 (lecture; English)

13. Kubovčiak, J., Kropáčková, L., Albrecht, T., **Těšický, M.**, Martin, J.F., Kreisinger, J.: Interspecific variability of gastrointestinal microbiota in passerine on the level of bacterial geni. The Zoological days, Brno, Czech Republic 2017 (poster; Czech)

12. Kreisinger, J., Kropáčková, L., **Těšický, M.**, Kubovčiak, J., Tomášek, O., Albrecht, T.: Gut microbiota differentiation between tropical vs. temperate passerine birds. The Zoological days, Brno, Czech Republic 2017 (poster; English)

11. Krajzingrová, T, **Těšický, M.**, Velová, H., Svobodová, J., Bauerová, P., Pechmanová, H., Albrecht, T., Vinkler, M.: The older, the better? The effect of ageing on condition-dependent traits in great tits (*Parus major*). The Zoological days, Brno, Czech Republic 2017 (poster; Czech)

10. **Těšický, M.**, Vinkler, M., Reifová, R., Velová, H.: Shared variability in selected innate immunity receptors in tits (Paridae), The Zoological days, Brno, Czech Republic 2017 (lecture; Czech)

9. **Těšický, M.**, Vinkler, M., Reifová, R., Velová, H.: Do have different tit species different alleles of immune genes? National conference of Czech Society for Ornithology, Mikulov, Czech Republic 2016 (lecture; Czech)

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