Charles University

Faculty of Science

Department of Zoology

Effects of peripheral inflammation on gene expression modulation in passerines and parrots

Efekt periferního zánětu na změny v genové expresi u pěvců a papoušků

Doctoral Thesis

Nithya Kuttiyarthu Veetil, MSc

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

Prague, 2023

Table of contents

Declaration of originality

I declare that this thesis or its substantial part has not been submitted to obtain the same or any other academic degree. I have written it independently based on the material cited in the text and in consultation with my supervisor and colleagues.

In Prague on 30.06.2023 Nithya Kuttiyarthu Veetil

PROHLÁŠENÍ

Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu. Tuto práci jsem zpracovala samostatně a uvedla jsem všechny použité informační zdroje a literaturu.

V Praze dne 30.06.2023 Nithya Kuttiyarthu Veetil

Statement of contribution

The results presented in the dissertation of Nithya Kuttiyarthu Veetil, MSc. are a joint work of the Laboratory of Evolutionary and Ecological Immunology and collaborative teams. The author statements included in the individual articles and manuscripts included in this thesis reflect my participation in the research described in this dissertation. All the literary works I consulted for this thesis have been appropriately cited.

PROHLÁŠENÍ O PODÍLU NA PUBLIKACÍCH

Výsledky prezentované v dizertační práci Mgr. Nithye Kuttiyarthu Veetil jsou společným dílem členů Laboratoře evoluční a ekologické imunologie a spolupracujících týmů. V každém článku či rukopisu obsaženém v této práci je uvedeno prohlášení o autorském podílu, které definuje mou účast na výzkumu popsaném v této doktorské práci. Veškerá literáratura, která sloužila jako základ pro tuto práci, byla řádně citována.

My contribution to all publications included in the doctoral thesis is as follows:

1) Michal Vinkler, Steven R Fiddaman, Martin Těšický, Emily O'Connor, Anna Savage, Tobias L. Lenz, Adrian L. Smith, Jim Kaufman, Daniel Bolnick, Charli Davies, Neira Dedić, Andrew S. Flies, M. Mercedes Gómez Samblás, Amberleigh Henschen, Karel Novák, Gemma Palomar, Nynke Raven, Kalifa Samake, Joel Slade, **Nithya Kuttiyarthu Veetil**, Eleni Voukali, Jacob Höglund, David S Richardson & Helena Westerdahl. "Understanding the evolution of immune genes in jawed vertebrates". *Journal of Evolutionary Biology* (2023).

   Information gathering and reviewing of the manuscript.

2) Voukali, Eleni, **Nithya Kuttiyarthu Veetil**, Pavel Němec, Pavel Stopka, and Michal Vinkler. "Comparison of plasma and cerebrospinal fluid proteomes identifies gene products guiding adult neurogenesis and neural differentiation in birds." *Scientific Reports* 11, no. 1 (2021): 1-16.

   Statistical analysis, data interpretation and reviewing of the manuscript.

3) **Nithya Kuttiyarthu Veetil**, Haniel Cedraz de Oliveira, Mercedes Goméz Samblas, Daniel Divín, Balraj Melepat, Eleni Voukali, Zuzana Świderska, Tereza Krajzingrová, Martin Těšický, Ferris Jung, Vladimír Beneš, Ole Madsen and Michal Vinkler. "Peripheral inflammation-induced changes in songbird brain gene expression: 3' mRNA transcriptomic approach". (Submitted to *Integrative and comparative biology*).

   Experiment conduction, RNA isolation, sample preparation for sequencing, bioinformatics analysis, differential gene expression, data interpretations, figure generation, and manuscript writing.

4) Divín, Daniel, Mercedes Goméz Samblas, **Nithya Kuttiyarthu Veetil**, Eleni Voukali, Zuzana Świderská, Tereza Krajzingrová, Martin Těšický et al. "Cannabinoid receptor 2 evolutionary gene loss makes parrots more susceptible to neuroinflammation." *Proceedings of the Royal Society B* 289, no. 1988 (2022): 20221941.

   Sample collection, experiment conduction, RNA isolation, bioinformatic analysis, figure generation and reviewing of the manuscript.

5) **Nithya Kuttiyarthu Veetil**, Amberleigh E. Henschen, Balraj Melepat, Rami A. Dalloul, Dana M. Hawley, Vladimír Beneš, James S. Adelman and Michal Vinkler. "Varying conjunctival immune response adaptations of house finch populations to a rapidly evolving bacterial pathogen". (Submitted to special edition of *Frontiers of Immunology)*

   RNA isolation, sample preparation for sequencing, bioinformatic analysis, differential gene expression analysis, data interpretations, figure generation, and manuscript writing.

Prague, 30 June 2023

Nithya Kuttiyarthu Veetil

doc. RNDr. Michal Vinkler, Ph.D.

List of publications

PAPER I

Michal Vinkler, Steven R Fiddaman, Martin Těšický, Emily O'Connor, Anna Savage, Tobias L. Lenz, Adrian L. Smith, Jim Kaufman, Daniel Bolnick, Charli Davies, Neira Dedić, Andrew S. Flies, M. Mercedes Gómez Samblás, Amberleigh Henschen, Karel Novák, Gemma Palomar, Nynke Raven, Kalifa Samake, Joel Slade, **Nithya Kuttiyarthu Veetil**, Eleni Voukali, Jacob Höglund, David S Richardson & Helena Westerdahl. "Understanding the evolution of immune genes in jawed vertebrates". *J Evol Biol*. 2023 Jun;36(6):847-873.

PAPER II

Voukali, Eleni, **Nithya Kuttiyarthu Veetil**, Pavel Němec, Pavel Stopka, and Michal Vinkler. "Comparison of plasma and cerebrospinal fluid proteomes identifies gene products guiding adult neurogenesis and neural differentiation in birds." *Scientific Reports* 11, no. 1 (2021): 1-16.

PAPER III

Nithya Kuttiyarthu Veetil, Haniel Cedraz de Oliveira, Mercedes Goméz Samblas, Daniel Divín, Balraj Melepat, Eleni Voukali, Zuzana Świderska, Tereza Krajzingrová, Martin Těšický, Ferris Jung, Vladimír Beneš, Ole Madsen and Michal Vinkler. "Peripheral inflammation-induced changes in songbird brain gene expression: 3' mRNA transcriptomic approach". (Submitted to *Integrative and comparative biology*).

PAPER IV

Divín, Daniel, Mercedes Goméz Samblas, **Nithya Kuttiyarthu Veetil**, Eleni Voukali, Zuzana Świderská, Tereza Krajzingrová, Martin Těšický et al. "Cannabinoid receptor 2 evolutionary gene loss makes parrots more susceptible to neuroinflammation." *Proceedings of the Royal Society B* 289, no. 1988 (2022): 20221941.

PAPER V

Nithya Kuttiyarthu Veetil, Amberleigh E. Henschen, Balraj Melepat, Rami A. Dalloul, Dana M. Hawley, Vladimír Beneš, James S. Adelman and Michal Vinkler. "Varying conjunctival immune response adaptations of house finch populations to a rapidly evolving bacterial pathogen". (Submitted to the special edition of *Frontiers of Immunology*)

Acknowledgement

I am very grateful for all my mentors who have guided me throughout my study life. When I look back, my PhD life started with mixed emotions, I was very anxious about stepping out of homeland and moving to Europe. But I was somehow able to quickly adapt to the new place. I received a lot of helping hands to cope up with the new atmosphere and for that I would first like to thank my supervisor and mentor, Michal Vinkler. His acceptance and supervision helped me to lay the steppingstone to my research career. He was always very willing to support and direct me throughout my entire PhD life.

My sincere gratitude to Ole Madsen and Haniel Cedraz de Oliveira, for guiding me during my stay in Wageningen university and always there to extend assistance during my whole PhD life. I would like to express my gratefulness to my former colleague Mercedes Gomez for all her support during my PhD days formally and informally. I would like to mention all my current and former lab members of Laboratory for Evolutionary and Ecological Immunology - Martin Těšický, Dan Divín, Tao Li, Katka Černá, Tereza Krajzingrová, Eleni Voukali, Zuzana Swiederska and Hana Velová for their support and easing my research journey. Special thanks to Anna Pospíšilová who aided me with workday challenges.

Special thank you to my non-group friends Rahul and Manon for the never-ending discussions and lunches. I am especially thankful to my Prague family members, Navyasree and Aswathi for making me feel the best in my lonely days and filling my tummy with the best homely foods.

I could not thank enough my family and my in-laws for their mental and moral support. They provided me with positivity, encouragement which was a guiding light for my journey throughout.

I am most thankful to my husband Balraj for his emotional support, for tolerating my tantrums and lifting me up whenever I am down. I could not have finished my PhD without his backing and inspiration. Thank you for believing and never letting me down.

Abstract (English)

Birds have well-defined roles in maintaining the ecological balance as predators, seed dispersers, nutrient cyclers, and pollinators making them an integral part of many ecosystems. Birds are often the flag-ship species and are important for wildlife preservation. Some of the avian populations are very well connected across the globe through their annual migration, increasing risks of epidemics of infections. Birds also face different levels of existence encounters in challenging living conditions like deserts and cold mountains. To cope with these diverse environments not only need physiological adaptations, but also a very well-equipped immune system, optimised to challenges common to the environment they inhabit. How well a host immune system responds to pathogens determines the overall fitness of the organism and its survival. Insight into the avian immune system functions is of great significance as birds are reservoirs of innumerable pathogens. They have been the primary source of several major epidemics' onset leading to worldwide human and animal fatalities (e.g., COVID-19, Avian influenza, or West Nile virus outbreaks). Similar to all living beings, avian hosts and pathogens are always in a continuous adaptational arms race. This coevolution of hosts and their pathogens forms the base of the host immune system evolution. Pathogens utilise diverse mechanisms to evade the host immune systems. However, the survival and success of all pathogens depend on how well they can colonize the host, evade the host's defences, multiply, and make the disease contagious. Switching of hosts by pathogens has led to the commencement of many drastic infection outbreaks.

Though extensive research efforts have been paid to avian species research, there are still missing gaps especially in less explored avian models and wild bird species. Studies on these species are significant as, unlike farmed poultry or laboratory models, wild birds are living in their natural habitats, with all natural factors acting on the hosts. Unlike inbred model organisms, wild models can provide a real-time dynamics description of natural immune regulation and adaptation in immunomodulation. The changes that arise in pathogens and hosts during infection can be detected through gene expression analysis of their tissue-specific transcriptomic profiles. This research offers a better insight into the ongoing immune regulation in the host-pathogen system and how it affects the host's fitness. The same pathogens may cause different immune responses in different host species. Each host has diverse modes of immune response regulation allowing it to overcome the pathogen attacks. Inflammation is one of the key immune systems responses allowing pathogen clearance in vertebrates. It is a highly controlled immune process, because of its potentially self-damaging nature. In my dissertation, to unveil the underlying mechanisms of immunogenetic variations and the evolution of immune genes, me and my colleagues revised the origin and diversification of immune genes, clarified different concepts related to the evolution of immune genes, and proposed different hypotheses using comparative approaches. Next, based on plasma and cerebrospinal fluid proteomes of parrots and chickens, we provided insights into avian adult neurogenesis, which is very important for the recovery from neuroinflammatory disorders. We also inspected the early inflammatory cytokine expression changes in the brain in passerines and parrots during peripheral inflammation using multiple bioinformatics approaches. The study revealed the gene loss of the neuroimmune gene regulator cannabinoid receptor 2 (*CNR2*) in parrots, which makes the parrots more susceptible to brain inflammation. Our study on zebra finches (*Taeniopygia guttata*) discovered controlled brain inflammation with a functional *CNR2* gene using a 3'end transcriptomic approach. We found evidence for ongoing adaptation in immune regulation in a wild disease model, the house finch (*Haemorhous mexicanus*) host, and *Mycoplasma gallisepticum* (MG) parasite. The results indicated how house finch populations with different coevolutionary histories with MG immunologically respond to MG isolates from two different evolutionary time points, providing evidence for immune regulatory mechanisms tackled by these birds to resist or tolerate the pathogen.

Abstract (in Czech)

Ptáci hrají významné role při udržování ekologické rovnováhy jako predátoři, roznašeči semen, cyklátoři živin a opylovači, což z nich činí nedílnou součást mnoha ekosystémů. Ptáci jsou často vlajkovými druhy a jsou tak důležití pro ochranu volně žijících živočichů obecně. Některé ptačí populace jsou globálně propojené v důsledku migrace, což je činí náchylnými k epidemiím infekcí. Ptáci také čelí různým existenčním hrozbám v náročných podmínkách prostředí, od pouští po studené hory. Aby se vyrovnali s těmito různorodými prostředími, potřebují nejen fyziologické adaptace, ale také velmi dobře vybavený imunitní systém optimalizovaný na patogeny běžné v prostředí, které obývají. Jak dobře imunitní systém hostitele reaguje na patogeny, určuje celkovou fitness a přežití jedince. Vhled do funkce ptačích imunitních systémů je velmi důležitý, protože ptáci jsou zásobárnou nesčetných patogenů. Byli primárním zdrojem několika velkých epidemií vedoucích k celosvětovým úmrtím lidí a zvířat (např. Covid 19, ptačí chřipka, virus západonilské horečky). Podobně jako všechny živé bytosti jsou ptačí hostitelé a patogeny vždy v neustálém adaptačním závodě ve zbrojení. Tato koevoluce hostitelů a jejich patogenů tvoří základ vývoje imunitního systému hostitele. Patogeny využívají různé mechanismy, aby se vyhnuly imunitnímu systému hostitele. Přežití a úspěch všech patogenů však závisí na tom, jak dobře dokážou kolonizovat hostitele, uniknout jejich obraně, množit se a způsobit nákazu nových hostitelů. Změna hostitele vedla u mnoha patogenů k počátku nových drastických infekcí.

Přestože výzkumu ptačích druhů bylo věnováno veliké výzkumné úsilí, stále existují v našem poznání mezery, zejména v méně prozkoumaných ptačích modelech a druzích volně žijících ptáků. Studie na těchto druzích jsou významné, protože na rozdíl od farmové drůbeže nebo laboratorních modelů žijí volně žijící ptáci ve svém přirozeném prostředí, kde na hostitele působí všechny přírodní faktory. Na rozdíl od běžně používaných inbredních modelových organismů, tyto divoké modely by mohly poskytnout popis dynamiky přirozené imunitní regulace a adaptací v imunomodulaci v reálném čase. Změny, které vznikají u patogenů a hostitelů během infekce, lze detekovat analýzou genové exprese pomocí tkáňově specifických transkriptomických profilů. Tyto studie poskytují dobrý přehled o probíhající imunitní regulaci v systému hostitel-patogen a o tom, jak ovlivňuje fitness hostitele. Stejné patogeny mohou u různých hostitelských druhů vyvolat různé imunitní reakce. Každý hostitel má vlastní způsob imunitní odpovědi umožňující mu překonat útoky patogenů. Zánět je jednou z klíčových reakcí imunitního systému sloužící k eliminaci patogenů u obratlovců. Jedná se o vysoce kontrolovaný imunitní proces, který má potenciálně sebepoškozující povahu. Abychom odhalili základní mechanismy imunogenetických variací a evoluce imunitních genů, v mém doktorském projektu jsme já a mí kolegové zrevidovali původ a diverzifikaci imunitních genů, představili různé koncepty evoluce imunitních genů a navrhli nové hypotézy. Dále jsme na základě proteomů plazmy a mozkomíšního moku papoušků a kuřat poskytli náhled na dospělou neurogenezi, což je proces velmi důležitý pro uzdravování z neurozánětlivých poruch. Pomocí různých bioinformatických přístupů jsme zkoumali časné změny exprese zánětlivých cytokinů v mozku u pěvců a papoušků během periferního zánětu. Studie odhalila ztrátu neuroimunologického regulačního genu kanabinoidního receptoru 2 (CNR2) u papoušků, kvůli které jsou papoušci náchylnější k neurologickému zánětu. Naše studie na zebřičkách (*Taeniopygia guttata*) s funkčním genem CNR2 popsala kontrolovaný zánět mozku pomocí 3'end transkriptomického přístupu. Našli jsme důkazy pro pokračující adaptaci v imunitní regulaci u modelu přirozeného onemocnění, divokého hostitele hýla mexického (*Haemorhous mexicanus*) a parazita *Mycoplasma gallisepticum* (MG). Výsledky ukázaly, jak populace hýlů s různou koevoluční historií s MG imunologicky reagují na izoláty MG ze dvou různých evolučních časových bodů, což poskytuje důkaz o adaptacích regulačních mechanismů imunity, kterými se tito ptáci přizpůsobují, aby získali rezistenci nebo toleranci k patogenu.

General Introduction

We are never alone. We thrive and survive with pathogens. This survival from the pathogens causing infections is one of the major selective pressures for all living organisms (Sironi et al. 2015). With the past works, in this host and pathogen research, we know that they always have conflicting interactions. These interactions are usually moulded by the constant interplay and sequences of adaptation and counteradaptation from the associated partners. This reciprocal selection can lead to coevolution, with constant variations in both host resistance and parasite infectivity, as stated by the Red Queen hypothesis. It also promotes genetic diversity, and influences a variety of life history traits (Papkou et al. 2019). Immune genes exhibit greater evidence for genetic diversity and adaptive evolution than others, due to the constant demand for the host immune systems to respond to the evolving pathogens (McTaggart et al. 2012). This continuous process eventually lead to the evolution of a large variety of immune defence mechanisms (Danilova 2006; Buchmann 2014).

Evolution of immunity occurs at different time spans i.e., from the profound temporal evolution of whole systems, to the evolution of variant pathogens and individuals within a species over the generations of a host species, to the positive selection on the frequency of lymphocyte clones in an individual at the time of infection (Kaufman 2010). One such evolved immune system innate immunity, which is the first defence line of organisms against pathogens has evolved over millions of years. It is composed of various immune cells and mediators, where many of which belong to the same gene families in most vertebrates (Medzhitov, Preston-Hurlburt, and Janeway 1997). When the innate immune system cells encounter with a pathogen or tissue injury the inflammatory response is activated (Newton and Dixit 2012). In more general terms, inflammation is the host immune system's response to injury or infection, characterized by redness, swelling, heat, and pain. But a long-term or persistent inflammation can affect the fitness of the host. Therefore, the immune system carefully manages the inflammation to maintain the host's health and fitness. We know that not all foreign particles can elicit a proper immune response. However, certain substances, such as pathogen-associated molecular patterns (PAMPs), danger-associated molecular patterns (DAMPs), fragments of dead cells, and toxins can well evoke the immune systems response.

PAMPs are conserved in most microbes (Thomma, Nürnberger, and Joosten 2011; Takeuchi and Akira 2010) while danger-associated molecular patterns (DAMPs) are typically intracellular molecules released during tissue damage (Vénéreau, Ceriotti, and Bianchi 2015). The host cells will recognize these PAMPs and DAMPs through the pattern recognition receptors (PRRs) (Amarante-Mendes et al. 2018). Upon their activation, PRRs send signals intracellularly which causes the initiation, production, and release of pro-inflammatory mediators, like cytokines, chemokines, and interferons (Kawai and Akira 2006). This induces the release of factors ensuring the recruitment of the leukocytes to the site of infection (L. Chen et al. 2018). The commonly triggered inflammatory signalling pathways are NF-κB, MAPK, and JAK-STAT (L. Chen et al. 2018). These mechanisms are highly conserved among vertebrates (Owen-Ashley and Wingfield 2007; Land 2020).

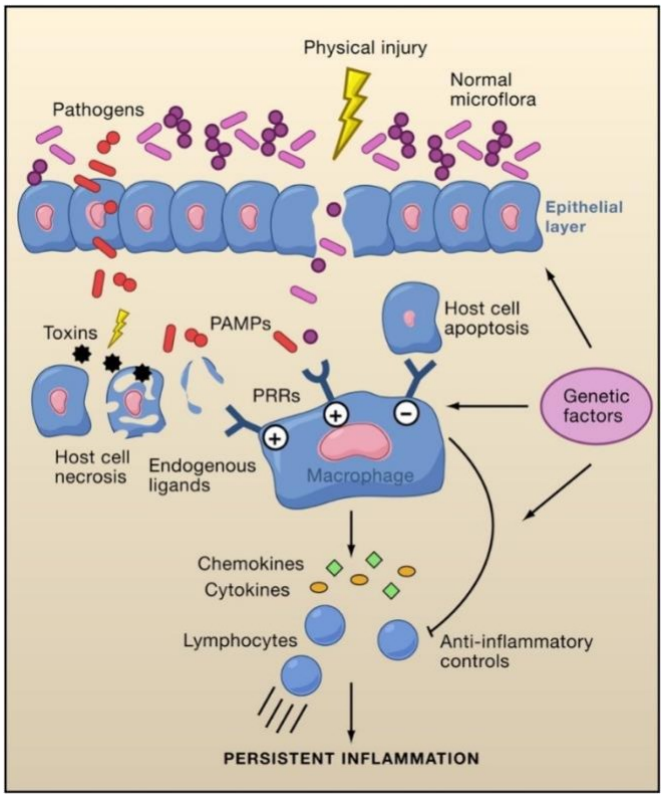

Figure 1: Key events occurring during an infection. The epithelial barrier is disturbed and leads to the increased exposure of resident inflammatory cells (macrophages, mast cells, dendritic cells) to microbes. PAMP and endogenous ligands are recognized by the macrophages through PRRs. Engagement of the PRRs triggers signalling pathways that lead to the release of chemokines and cytokines, the recruitment and activation of lymphocytes, and the propagation of chronic inflammation. Courtesy (Karin, Lawrence, and Nizet 2006) (License-CC BY-NC-SA 3.0)

As mentioned above, a properly balanced inflammatory response is very important, as this offer a wide range of protection against infections and coordinate long-term adaptive immunity toward specific pathogens (Xiao 2017). It also eliminates harmful stimuli and promotes the healing process by initiating tissue repair and recovery (L. Chen et al. 2018). Hence, inflammation is a defence mechanism that is vital to health (Nathan and Ding 2010). Usually, during acute inflammatory responses, all the immune genes involved collectively contribute to the elimination of the injury or infection and re-establish tissue homeostasis. However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases (Zhou, Hong, and Huang 2016). The role of inflammation is crucial when the host's inflammatory response, rather than pathogen toxins, is primarily responsible for host damage. For example, in tuberculosis caused by *Mycobacterium tuberculosis*, bacteria can persist in the host organism for decades leading to chronic inflammation. Severe inflammation leads to lung liquefaction, causing a respiratory capacity loss for the host and facilitating pathogen transmission through infection droplets (Nathan and Ding 2010). In the above example, the chronic inflammation occurring in a specific tissue leads to the dysfunction or failure of the entire organ. But the effect of chronic inflammation is more wide and systemic causing damage to different parts of the host including the brain. Persistent peripheral inflammation and innate immune system activation are considered major trigger leading to acute neuroinflammatory disease progression (Träger and Tabrizi 2013). This brain

inflammation further leads to stress-related behaviour changes (Ravi, Miller, and Michopoulos 2021) in the organisms such as sickness behaviours like sadness, fatigue, decreased food intake, altered sleep, and social-behavioural isolation which may be critical for survival during infection or injuries (R. Straub et al. 2010; R. H. Straub 2017; Furman et al. 2019). Though the immediate effect of neuroinflammation is sickness behaviour, it can also alter neurogenesis (Sung et al. 2020). Neurogenesis is an important factor in establishing and preserving cognitive function and repairing brain cellular damage caused by aging and brain disorders (Poulose et al. 2017). During a lifetime, adult neurogenesis has a significant role in both normal brain functioning and regulating brain-related illness (Zhao, Deng, and Gage 2008), especially, the role of cerebrospinal fluid (CSF) in mediating the adult neurogenesis (Zappaterra and Lehtinen 2012; Fame and Lehtinen 2020; Sawamoto et al. 2006; Lehtinen et al. 2011; Bachy, Kozyraki, and Wassef 2008; Villeda et al. 2011).

The key step in studying neuroinflammation or inflammation and immunity is selecting the appropriate model organism. Most of the inflammation and immune-related studies have been conducted in model organisms like rodents including neuroimmune studies (Papadopoulos et al. 2014; Cardinal et al. 2021; Dong et al. 2021; Ellenbroek and Youn 2016). Model organisms are vital for biological research as they facilitate researchers to study diseases quicker, cheaper, and in a wide range. But the use of model organisms has several limitations. For example, being under laboratory conditions with standardised genomes, they mostly lack genetic variation (Magalhães 2015), making them often not suitable for studies related to the evolution of immune genes. Rodents have limited cognition abilities and lack some other traits characteristic to humans, such as day activity that is shared with birds (Crystal 2012; Bateson and Feenders 2010a). Domestic chicken *(Gallus gallus*) has served as an important classical avian model organism for many inflammations, developmental and neural studies (Dal Pont et al. 2021; French et al. 2020; Xiaolong Zhang et al. 2022; Flores-Santin and Burggren 2021; Voukali et al. 2021). But to be noted, the modern chicken in its many forms is the result of artificial selection stronger than nearly any other domesticated animal (Flores-Santin and Burggren 2021). Recent evidence shows that the neural densities of chickens are much lower than other evolutionarily derived birds like parrots and passerines (Stapley et al. 2008; Wirthlin et al. 2018), indicating the limits of this model organism for neuroinflammation studies. Studying other species within the vast biodiversity, including less studied models and free-living animals with unexplored traits living in their natural habitats, could help to overcome some of the above shortcomings (Magalhães 2015) and yield additional insights into the evolution of inflammation, health, condition, and immunity.

There is one more additional advantage of using non-model organisms in eco-immunological research, as we can assess the immune responses linked with host fitness by together considering the environmental and genetic variability (Demas, Nelson, and Nelson 2012). The passerines (Passeriformes) and parrots (Psittaciformes) are some of the common non-model organisms used in the field of eco-immunology (Hickman et al. 2017; Griffith et al. 2021; Ohmer et al. 2021). Though they are cognitively more advanced than usual laboratory models, often there is a shortage of information about their basic biology. Phylogenetic evidence suggest that parrots are the closest relatives of passerine birds which made them significant models in many comparative studies (Bateson and Feenders 2010b) such as evolutionary studies, brain function analysis, immunological research, and development of molecular markers (Hains et al. 2020; Warren et al. 2010). They are known for their exceptional cognitive abilities and complex social interactions similar to most advanced vertebrates (Medina-García, Jawor, and Wright 2017) and have shown sickness behaviours like lethargy during pathogen infection (Hess, Bartick, and Hoefer 1998). Veterinarians have noticed and diagnosed signs of depression in parrots, such as anxiety, apathy, overeating, indifference, and self-harm (plucking feathers) (van Zeeland et al. 2009; Péron and Grosset 2014; J. Chen et al. 2020). Additionally, both parrots and passerines serve

as ideal subjects for biological experiments due to their suitability for captive breeding. They can be well maintained in cages, exhibit excellent breeding capabilities, and have moderate lifespans (Young et al. 2012). Also, their advanced cognitive abilities enable researchers to establish connections between physiological processes, neuronal structures, and behavioural patterns (Olkowicz et al. 2016; Emery 2006). Moreover, the availability of a complete sequenced genomes for some of the parrot and passerine species, also makes them an efficient model in comparative research at both inter and intraspecific levels.

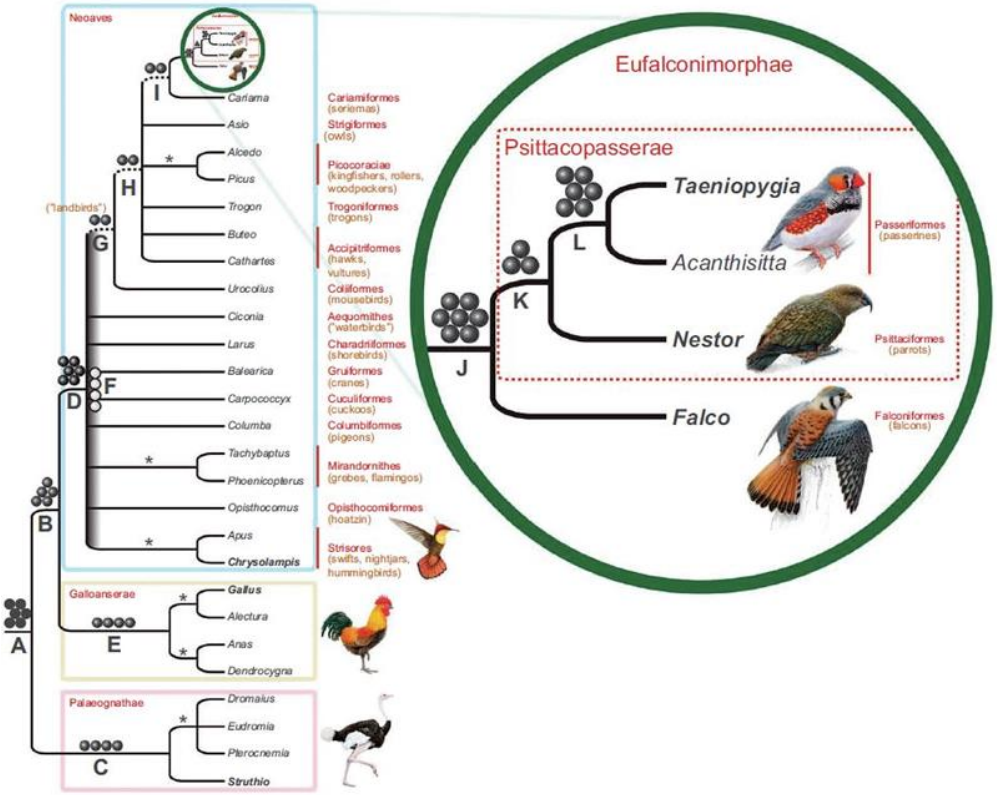

Figure 2: Retroposon evidence for the early branching events in the avian tree of life. Black filled circles (branch A) are bird-specific retroposon insertions. Dark grey balls represent retroposon presence/absence markers that are congruent with one another. Light grey balls on grey gradient (label F) are retroposon markers that were probably inserted at the very beginning of the neoavian radiation. This schematic diagram shows the relatedness of passerines and parrots, and also how distant is gallus from these species Courtesy (Suh et al. 2011) (License-CC BY-NC-SA 3.0)

In addition to the above-mentioned advantages of using these birds as model organisms, there is further additional immunological advantages when studying naturally occurring wildlife bird-pathogen model systems. These bird hosts offer a clearer understanding of real-life immune system functioning. It is also important to study such systems due to the frequent occurrence of new zoonotic infections that are transmitted from wildlife to humans (Bloom, Black, and Rappuoli 2017; Cunningham, Daszak, and Wood 2017). The primary limitation associated with utilizing the wild host-pathogen model is the challenge of obtaining access to these systems and conducting practical assessments of immune responses. Not only in birds, overall, in vertebrates there are very less such host-pathogen systems studied. Some examples of such studies in vertebrates are in amphibians (Voyles et al. 2018) and bats (Langwig et al. 2017) challenged by fungus or rabbits encountered by myxoma virus (Alves et al. 2019).

One such available model system is the house finch (*Haemorhous mexicanus*) and *Mycoplasma gallisepticum* (MG). This model system has undergone coevolution for nearly three decades, with intermittent disease outbreaks spanning across the US from east to west, providing compelling evidence of a continuous arms race of survival and evolution.

Once we have identified the right model organism to study the immunological responses, the next major challenge is identifying the appropriate methodology to identify and connect the underlying molecular mechanism with the immune gene response. The omics technology is one of the most modern and accurate methods today available to connect the gaps in the biological field. Transcriptome profiling by RNA-based next-generation sequencing (RNA-seq) is one of the most extensively used omics technology in immunological research. As budgerigars and zebra finches have fully sequenced genomes, the bioinformatic analysis of RNA-seq data is much easier and more accurate. The genome of these birds could even serve as a reference genome for the transcriptomic profiling of birds from the same family. In the last decade, RNA-seq price had dropped significantly. The relatively low cost and increased sensitivity of RNA-seq compared to other sequencing methods make it a cost-effective option for numerous scientific researches (Z. Wang, Gerstein, and Snyder 2009). Easier, cheaper, and faster alternatives for RNA-seq technologies also have been developed in recent years like QuantSeq, which generates highly strand-specific NGS libraries close to the 3′ end of polyadenylated RNAs (Moll et al. 2014)(including mRNA and most the lncRNAs). As the use of RNA-seq is for abundance estimation at the gene level and for differential transcript expression under various circumstances, 3' end sequencing by QuantSeq approach can be an appropriate choice.

The QuantSeq approach is a reliable method for gene expression analysis and has better contributed to the understanding of transcriptomic profiling even in less explored species (Corley et al. 2019; Moll et al. 2014; Jarvis et al. 2020; Ma et al. 2019; Vo et al. 2021). So, here we have opted QuantSeq for the estimation of differential gene expression in our model organisms which was later confirmed by RT-qPCR. In this thesis, we aim to describe the investigated immunogenetic variations and the evolution of immune genes, including the evidence for adult neurogenesis using different omics approaches. The research highlights the use of the QuantSeq approach as an alternative to the classical RNA-seq method to identify the gene expression change in both the avian brain and periphery induced by sterile inflammation. We describe the early inflammatory cytokine expression changes in zebra finch and parrots and how these variations in immunity induce different behaviour and physiological changes in these species. And finally, we explored the immune regulation in conjunctiva tissue of the wild host-pathogen model, in four different house finch populations infected with MG strains from two distinct evolutionary time points, revealing the diverse immune response and immune regulatory mechanisms opted by the birds against the pathogen. Overall, my research contributes to a better understanding of immunogenetics, immune gene evolution, and neuroinflammation in different avian species. The following aims provide a more detailed explanation of the research objectives.

General aims

1) **How variation in immunity arise among different species? What are the underlying evolutionary molecular mechanisms that shape immune system regulation?**

   Host immunity is constantly adapting to maximize defence efficiency against diseases within a given environment, driven by co-evolution with pathogens. More thorough research into how immunogenetic variation impacts the diversity of immune responses seen across domestic and wild animal species is now possible due to recent advancements in analytical methods and bioinformatics approaches. We reviewed the present understanding of the evolution of immune gene diversity in vertebrates. Through comparative approaches, we put forward novel hypotheses for future experimentation (**PAPER I**).

2) **Does neurogenesis occur in adult birds?**

   Neurogenesis plays a crucial role in maintaining cognitive function and facilitating the repair of damaged brain cells associated with aging, brain inflammation and, brain disorders. Employing a proteomic approach, our study sought to investigate the interspecific variation in CSF and plasma proteins that contribute to the physiological regulation of adult neurogenesis in diverse species of highly cognitively advanced avian organisms (**PAPER II**).

3) **What is the effect of peripheral inflammation on immune homeostasis of avian brain?**

   Inflammation in the periphery triggers cytokine signalling that acts systemically to affect distant organs. Inflammation may be propagated across the blood-brain barrier to induce neuroinflammation. Inflammation in the brain leads to sickness behaviour in some birds including social withdrawal symptoms. We examined gene expression changes in the brain of parrots and a passerine and evaluated the impact in the brain of two bird groups using genomics and transcriptomics approaches. We also revealed that the 3'end transcriptomic strategy is a reliable method for identifying avian brain inflammation (**PAPER III and PAPER IV**).

4) **What is the immunological mechanism of tolerance to infection that evolves in the host in response to evolution of increased virulence in a recently emerged pathogen?**

   The interaction of the house finch and MG has developed into a significant natural model of coevolution between a host and an evolving pathogen, allowing researchers to gain insight into vital aspects of disease ecology and evolutionary biology. However, our knowledge of the crucial immunological features of the house finch response to MG remains limited. We described the variations in the immunological response among the different house finch populations (differing coevolutionary history with MG) against the original and evolved isolates of MG using a 3' end transcriptomic approach (**PAPER V**).

General Methods

Within this section, I attempted to present a broad overview of our approaches and highlight their specificities, rather than providing intricate details that can be readily accessed in the individual manuscripts.

Model Organisms

I used birds belonging to Psittaciformes (parrots) and Passeriformes (songbirds) orders as models for my research.

Psittaciformes (parrots)

Parrots (Psittaciformes), comprising approximately 400 species, are widely distributed across diverse ecosystems. Notably, parrots possess a high degree of breeding adaptability, making them easily breedable in captivity and popular among hobby breeders (Tella, Blanco, and Carrete 2022). The main diet of parrots typically consists of seeds, fruits, nectar, and pollens and occasionally some small animals. They are distributed in tropical and subtropical climatic zones. Molecular studies indicate that parrots evolved in Gondwana about 59 million years ago (Tavares et al. 2006). They are known for their exceptional cognitive abilities and complex social interactions (Medina-García, Jawor, and Wright 2017; Wirthlin et al. 2018). Nearly 30% of parrots species are threatened with extinction, with another ca. 15% classified as nearly threatened (Vergara-Tabares et al. 2020; Barbosa et al. 2021; Chan et al. 2021). There is still lack a of comprehensive studies on parrot diseases. Though man-kept parrots are one of the most popular pet birds, parrots have shown social problems like abnormal and repetitive behaviours including feather damage and stereotypy which are indicative signs of weakened psychological health during stress conditions (Cussen and Mench 2015). In our research studies (**PAPER II and PAPER IV**), we used different parrot species (covering several different clades of parrot phylogeny) to describe adult neurogenesis using proteomic approach and neuroimmune regulations during peripheral stimulation using genomic and transcriptomic approaches.

Figure 3: Budgerigar (Courtesy: Martin Těšický)

Passeriformes (songbirds)

Zebra finch

The order of passerine birds (Passeriformes) is an immensely diverse group, containing nearly 60% of all living bird species. Passerines have evolved on the southern supercontinent Gondwana (Ericson et al. 2014). Zebra finch (*Taeniopygia guttata*), one of the popular pet birds from the estrildid finch family is a small-sized bird and mainly feeds on grass seeds (Zann et al. 1995). Previous research has proved that zebra finch is an excellent subject for the analysis of neurological processes (Oksche et al. 1963). Studies show that they are an exceptional model organism for brain-related experiments (Brainard and Doupe 2002). For example, they share a number of cognitive and physiological adaptations convergent to humans which are absent in rodents (Spierings and Ten Cate 2016). Since zebra finches retain their learned capacities regardless of the neural turnover lined with adult neurogenesis, this model taxon could importantly aid the future investigation in treatments for brain-related diseases (Barnea and Pravosudov 2011). Zebra finches currently used for laboratory research have been obtained from the domestic populations accessed through pet shop supplies (Forstmeier et al. 2007). This is an appealing change from the classic model organisms that are usually available as commercial strains (Gasch, Payseur, and Pool 2016). Previous research shows that zebra finches respond rapidly to trauma and can control the natural inflammation that happens in the brain during injury (A. L. Pedersen, Nelson, and Saldanha 2016), which is different in parrots where the birds are prone to neuroinflammation. In **PAPER III**, we aimed to describe the biological phenomenon of systemic inflammation regulation in zebra finches by identifying the inflammatory regulation occurring in the bird brain during peripheral stimulation.

Figure 4: Zebra finches (Courtesy: Hana Velová)

House finch

House finch (*Haemorhous mexicanus*) belongs to the family of true finches, Fringillidae. This is a moderate-sized finch. They feed mainly on grains, seeds, berries, and weed seeds. Occasionally they consume small insects. They are native to western North America and were later introduced to the

eastern half of the continent and Hawaii islands after they were released into the wild by pet traders (Able and Belthoff 1998). Over the subsequent decades, this eastern population of house finches expanded its distribution across half of the continent and the population size increased exponentially (Q. Zhang et al. 2014). Their usual breeding habitat is urban and suburban areas across North America. At ecological and evolutionary scales, host-pathogen interactions are dynamic. For instance, the house finch and bacterium *Mycoplasma gallisepticum* (MG), are part of a host-pathogen system which can provide deeper understanding of the adaptations in immune regulations. MG is an economically important poultry pathogen creating alarming threat also to free-living birds (Messa Júnior et al. 2017). In the 1990s one lineage of MG switched from galliform birds to house finches as its novel host (Dhondt, Tessaglia, and Slothower 1998). As a result of the epidemic caused by the MG which emerged from the Washington D.C area in 1994 and gradually spread over the eastern region, house finch populations in the eastern US and Canada declined almost by 50% between the time period of 1994 and 1997 due to severe conjunctivitis (Ley, Berkhoff, and McLaren 1996; Hawley et al. 2013a; Hochachka and Dhondt, n.d.).

The evolutionarily original MG isolates (e.g. the isolate VA1994) cause in the present hosts of the co-evolving populations milder disease with rapid recovery, while the evolved MG isolates (e.g. the isolate NC2006 or VA2013) tend to induce stronger pathology with prolonged healing periods (Hawley et al. 2013b). At the same time, the host appears to evolve in response to MG pathogenic pressure. There is inter-individual variability among house finches in their resistance to MG and some house finches recover more rapidly from the MG infection than others (Hill and Farmer 2005). House finch populations differing in the length of their contact with the pathogen apparently differ in their inflammatory immune responses to MG (Adelman et al. 2013). The house finch-MG host-pathogen model system is currently unique in avian evolutionary ecology given the amount of evidence already obtained and also the wealth of variable biological material available for future experimental work aimed at understanding the means of adaptations in this arms race. In our **PAPER V**, we examined natural populations of four different house finches infected with two different strains of MG (the original 1994 isolate and a more evolutionary derived one collected in 2013) for understanding the early immune gene regulations exhibited by the host population. Analysing gene expression using transcriptomics approach, we have shown how each house finch population (which differs in their contact history with MG) regulates the immune response against the different strains of the pathogen.

Figure 5: House finch (Courtesy: Bonnie Fairbanks Flint)

Summary of the key methods

Bird collection and experimental design

Different bird groups used for the experiments in my thesis are either collected from local hobby breeders (zebra finches and parrots) or directly from the wild (house finches) with the help of our collaborators. The birds had access to food and water ad libitum and were kept under a controlled light/dark cycle with a regulated temperature. In zebra finches and parrots, the experimental bird groups were subjected to intra-abdominal administration of LPS (Escherichia coli O55:B5; Sigma-Aldrich, cat. no. L2880) at a dosage equivalent to 6 µg per gram of the body weight. The control group, on the other hand, received an injection of sterile Dulbecco's phosphate-buffered saline (Sigma-Aldrich, cat. no. D5652). The LPS dosage was determined based on previous investigations involving other avian species of similar size, where it induced a measurable non-specific immune response (Wegmann, Voegeli, and Richner 2015). For the house finches, juvenile birds were captured from the wild using mist nets and feeder traps from in Blacksburg, Virginia; Ames, Iowa; Tempe, Arizona; and Waianae, Oahu, Hawaii (between June-September 2018). Finches exhibiting any clinical signs of MG infection at the time of capture were immediately released. Upon arrival, the birds underwent a period of acclimation and quarantine. The birds were subjected to inoculation with either a control treatment (sterile Frey's medium) or a high dose ($7.5x10^6$ CCU) of the lower-virulence state.

All the birds were euthanized using CO_2 and collected from different tissue panels. I have tested multiple RNA isolation kits for the extraction of RNA from brain tissue. The quality and quantity of extracted RNA were analysed using Nanodrop and Bioanalyzer. The results confirmed that Roche High Pure RNA Isolation Kit outperformed the others in terms of quality. The RNA was isolated from the brain and multiple peripheral tissues and the eluted RNA was stored in the freezer at -80ºC.

Library preparation for QuantSeq approach

QuantSeq uses total RNA as input, therefore no prior poly(A) enrichment or rRNA depletion is required unlike the usual RNA-seq (Moll et al. 2014). Because QuantSeq sequences a smaller portion of the transcript and produces only one read per transcript, it requires less sequencing capacity than the standard RNA-Seq (Corley et al. 2019). As the protocol does not have a ligation step, it is possible to quickly produce amplicons (DNA fragments that are generated through the amplification of a specific region located near the 3'end of a target molecule) made from mRNA 3′ ends (Bhat et al. 2021). OligodT priming is the first step in library preparation and the primer has Illumina-compatible linker sequences. When the first strand synthesis is completed, the RNA is removed. The second strand synthesis consists of random priming and DNA polymerase. The random primer also has Illumina-compatible linker or adapter sequences. No purification step is necessary between the first and second strand synthesis. After the second strand synthesis, a magnetic bead-based purification step is done. This is followed by library PCR amplification and cluster generation and sequencing (Moll et al. 2014) with the Illumina (Hiseq 2500) instrument. Reduced library complexity of the 3' end sequencing approach makes it possible to properly identify transcript abundance with lesser sequencing depth, enabling larger levels of multiplexing while keeping sensitivity comparable to that of traditional mRNA sequencing methods (Bhat et al. 2021). The library preparation and transcriptome sequencing were carried out at the European Molecular Biology Laboratory (EMBL), Heidelberg. For each library, 80-base-long single-end reads were generated.

Figure 6: Schematic diagram of mRNA indicating the 3′ region from which QuantSeq reads are derived and RNA-seq reads come from the entire transcript. Courtesy (Corley et al. 2019) (License-CC BY-NC-SA 3.0)

A classic RNA-seq workflow usually comprises the following steps: raw data pre-processing, read mapping, expression quantification, and differential expression analysis. Before data pre-processing, RNA-seq data may first be submitted to quality control (QC) of the raw data. The next step involves the adapter trimming followed by read mapping to the reference genome. Then, the gene level expression is estimated and finally, differential gene expression is estimated (Yang and Kim 2015). There are several numbers of tools available for the analysis and the user's decisions on the reference genome, annotation tools, and associated parameter settings at each stage of the analysis will have a significant impact on the analysis's outcome.

Bioinformatics interpretation and differential gene expression analysis

I tested two different pipelines, namely BAQCOM and Bluebee on the sequenced data. After evaluating the results, I selected the BAQCOM pipeline for the remaining analysis. Following the de-multiplexing of the sequenced data, the raw data QC is performed using the FastQC tool(Andrews 2010). This analysis displays the quality profile of the sequenced data. The next step is the adapter removal. It removes the ligated adapters to samples during library preparation. Trimmomatic tool is used for the same (Bolger, Lohse, and Usadel 2014). Next, the sequenced reads are aligned to the reference genome using the STAR aligner (Dobin et al. 2013). The reference genome and annotation files are downloaded from the online bioinformatic resource, Ensembl (Howe et al. 2021). For the expression quantification, the featureCounts, (Liao, Smyth, and Shi 2014) tool was used for counting reads generation after the alignment with the reference genome. Finally, the differential gene expression was estimated using the DESeq2 package from R Bioconductor. The sequencing results were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA). Gene functional annotations (gene ontology, GO) were assigned using the Ensembl BioMart (Smedley et al. 2015) with the bird reference, manually supplemented with Uniprot (The UniProt Consortium et al. 2021) annotations. The GO terms for unannotated genes were attributed by finding orthologous genes in the chicken or human reference using gprofiler (Raudvere et al. 2019).

To understand changes in expression in lowly- expressed genes, we adopted the following approach to reveal more subtle differences compared to the transcriptomic approach. We first divided the total number of reference (cytokine)-aligned reads by the total number of reads in the sample to normalize the expression data in the remaining target genes (Cn). Then, we multiplied each of the normalized read counts by 10 million to scale the data (approx. 10 million was the average number of reads per sample in our dataset). Relative differential gene expression = $(Cn \times 10^6)_{Treatment}/((Cn \times 10^6)/N)_{Control}$. The

cytokine expression was calculated as the scaled-normalized number of reads per treatment individual divided by the mean scaled-normalized number of reads in all the control birds.

The cheaper cost of sequencing using QuantSeq allowed us to include more samples in the research, which is its greatest benefit. This becomes especially crucial when working with less common model organism because individual genetic variation might also result in erroneous differences in DGE between control and treatment groups. We validated the DGE of a few genes using RT-qPCR (TaqMan probe-based assay).

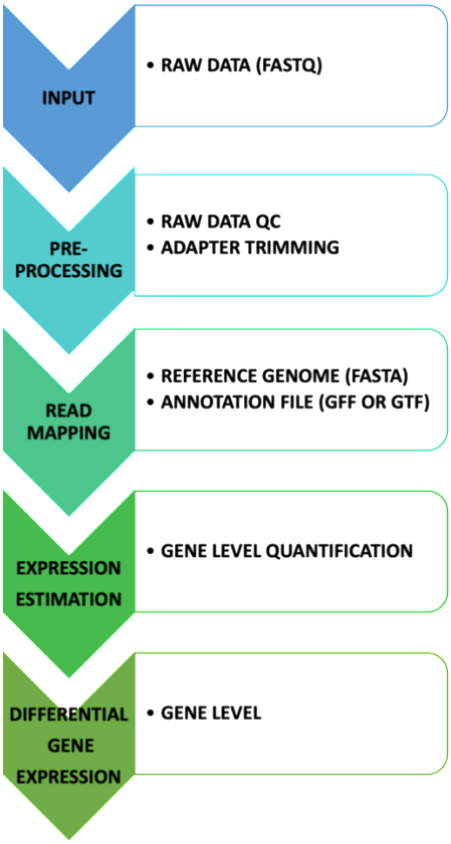

Figure 7: Workflow of RNA-seq data (reference-based) analysis using bioinformatics tool at gene level. This schematic diagram provides an illustration of the BAQCOM pipeline we used for running the bioinformatics analysis, quantification of the read counts and differential expression analysis at the gene level.

Proteomic data analysis

A total of seven chickens, five budgerigars, and five cockatiels were used to collect CSF and plasma samples. The animals were collected from local hobby breeders and were kept in the departmental animal facility. Birds were euthanized using CO_2. The collected CSF and plasma were stored at -80°C. For the isolation and purification of proteins from the above samples, ProteoSpin detergent-free total protein isolation kit (Norgen Biotek, Thorold, ON, Canada) was used. The isolated proteins were stored at -20°C. Protein precipitation was carried out using acetone and the samples were dried for 30 minutes at 37°C. Subsequently, the samples were treated with trypsin and incubated at 37°C. Mass spectrometry analysis was carried out for protein detection (nLC − MS/MS analysis). Proteomic data was analysed using MaxQuant software (Cox et al. 2014). R software was used for statistical and bioinformatic analysis. Differential protein expression was estimated using the Differential Enrichment analysis of the

Proteomics data (DEP) package (Xiaofei Zhang et al. 2018) in R. The package Interacting Gene Retrieval (STRING 11.0; http://string-db.org) (Mering et al. 2003) web-tool was used to analyse and construct the PPI network of the common group of proteins that were over-represented in CSF compared to PL (adjusted p-value cut off (FDR) 0.05, identified by the t-test analysis). We used the online ShinyGO (v0.61) (Ge, Jung, and Yao 2020) tool to identify the GO terms of biological functions that are significantly overrepresented in CSF compared to blood plasma network visualization. ShinyGO can provide a graphical image of enrichment, pathway, gene features, and protein interactions (Ge, Jung, and Yao 2020). Gene set Enrichment Analysis (GSEA) was performed. This is a potent tool for interpreting gene expression data (Subramanian et al. 2005). When comparing CSF to plasma, the analysis based on GO biological functions classification and pathway enrichment indicated differential representation in proteins related to neuronal function. Using annotations from humans, all proteins were ranked according to the correlation between their abundance and the classification (CSF or Plasma) (Human Gene Symbol with Remapping MSigDB.v7.2). As previously advised, we employed the GOs analysis of biological processes with nominal p values 0.05 and adjusted p-values 0.25 (Subramanian et al. 2005).

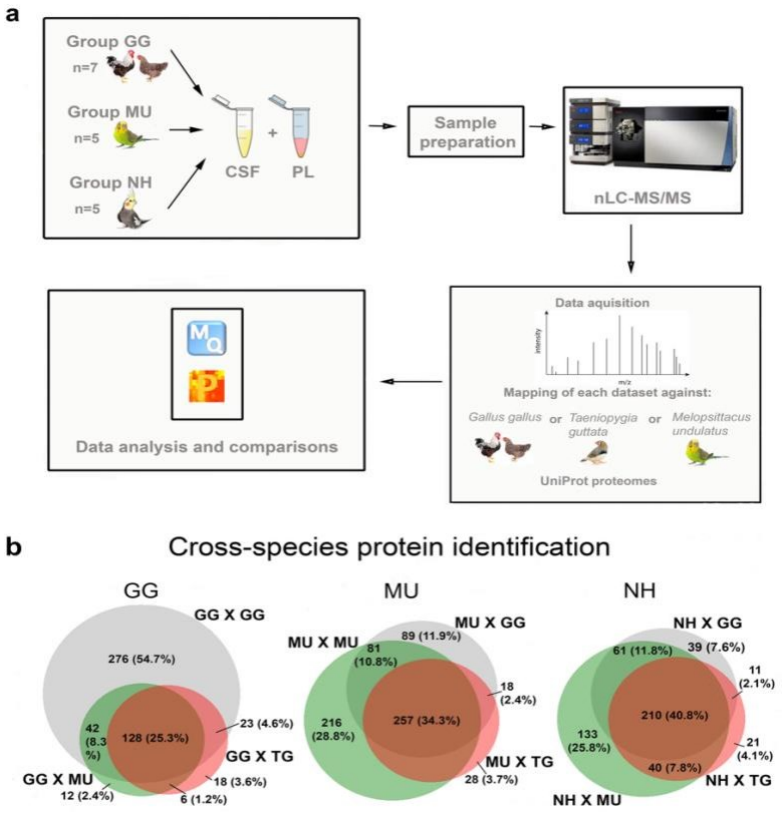

Figure 8: Comparison of the nLC-MS/MS cross-species protein identification success in both cerebrospinal fluid (CSF) and plasma (PL) samples of chickens (GG), budgerigars (MU) and cockatiels (NH), after mapping to three selected avian reference proteomes GG, MU and Taeniopygia guttata (TG). (a) The overview of the study design and mapping approach is schematically illustrated. (b) Venn diagrams show the proportions of proteins identified based on the different reference proteomes of GG (grey), TG (red) and MU (green) for GG, MU and NH (BioVenn, http://www.biovenn.nl/) (Voukali et al. 2021) (License-CC BY-NC-SA 3.0).

General results and discussion

Coevolution between hosts and pathogens shapes the immune systems in all organisms. Emerging infectious diseases apply novel and powerful selection pressures on hosts (Vinkler et al. 2018). Evolutionary immunology aims to describe the immune diversity among and across species resulting in variations in immune regulation (Barreiro and Quintana-Murci 2010). Nature exhibits a wide array of defences in hosts against parasitism, encompassing immune mechanisms like resistance and tolerance, as well as behavioural strategies such as social avoidance and mate choice. These defence strategies coexist with a multitude of parasitic attributes, including virulence, chronicity, and acuteness, various life cycles involving one or multiple hosts, and diverse transmission mechanisms such as airborne, environmental, social, and sexual routes. Understanding the reasons behind this remarkable diversity necessitates an examination of how different traits in hosts and parasites have evolved in response to one another. Host-parasite coevolution involves hosts adapting to avoid or tolerate infections, while parasites reciprocally counter-adapt to evade or overcome host defences (Buckingham and Ashby 2022). Several evolutionary forces impact immunogenetic diversity. Immunity and immune regulations are controlled by numerous genes which have a direct and indirect role in defence mechanisms. About 10 % of the genes in the vertebrate genomes are involved in immunity (Vinkler et al. 2023). Immune related genes are one of the most rapidly evolving genes within animals (Behrman et al. 2018) and the use of common laboratory model species may not be sufficient to achieve the rightful information about the complex mechanisms underlying vertebrate immune system (Mestas and Hughes 2004). Thus, comparative research is necessary to reveal functional immunological variation at both inter and intraspecific levels. The availability of genomic data alone does not provide all the necessary insights, but recent developments in immunogenomics (like functional association studies) offer ways to explain this variability (Holt 2015). We need to develop specific hypotheses and verifiable predictions that can explain this immunogenetic variability to deepen our understanding.

When researching host-parasite coevolution, most researches were concentrated on the candidate gene method, focusing on a few well-known candidates, like TLRs (Toll-like receptors) or MHC (Major Histocompatibility Complex) (Borghans, Beltman, and De Boer 2004; Těšický and Vinkler 2015; Khan et al. 2019). It is now apparent that investigating MHC alone is unable to account for the majority of adaptive variation against infectious illnesses (Acevedo-Whitehouse and Cunningham 2006). Although the majority of our conclusions regarding evolutionary immunology have derived from the MHC, the MHC is not a very feasible model for all immune studies due to its complex and instant evolution. Usually in the pursuit of answering research questions, we usually search for model genes but with the widespread adoption of genomics and transcriptomics, it is now possible to discover novel candidates for immune genes. So how can you discover the right immunity gene among thousands of others? To a certain extent, using different methods and model organisms, we try to answer this question in the following research papers.

In **Paper I** my co-authors and I reviewed the current knowledge on the evolution of immune genes in vertebrates. We summarised how to use genomic or transcriptomic approaches to search for potential genes. We reviewed model host-parasite systems tracked for host immune gene evolution. First, we introduce the immunome concept, a framework for hierarchical characterising genes involved in immune defence: (i) the core immunome: genes whose primary (and in many cases, only) physiological function is in the recognition of, and/or response to, pathogens; (ii) the peripheral immunome: genes with a clear immunological role, but which also contribute to non-immune physiological function; and (iii) non-immune resistance genes (NIRGs; which could be called the accessory immunome or even the resistome). The core immunome's genes are typically also examined for other significant evolutionary

events, such as convergence and parallel/coordinated evolution (Wutzler, Foerster, and Kempenaers 2012; Pavlovich et al. 2018; Świderská et al. 2018; Těšický et al. 2020). Second, we focus on how different modes of selection can be observed in different groups of immune genes and propose hypotheses to explain these differences. We then provide an overview of the methods to study the evolutionary heterogeneity of immune genes at different evolutionary levels. Finally, we discuss some of the current evidence for how specific pathogens influence the evolution of different groups of immune genes. In this review paper, we highlight that evolutionary genetics must be combined with other types of data to get a better understanding of immunological regulations involved in host immune defence evolution. The result interpretation through integrated analyses is critical for the advancement of this field. This is best accomplished through the collaborative participation of scientists from various backgrounds, skills, and perspectives.

Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood (Ziv et al. 2006). The central nervous system (CNS) and the immune system are both intricate and highly organized systems that regulate the entire body, with both sharing certain common features in developmental mechanisms and operational modes (Morimoto and Nakajima 2019). The function of the central nervous system (CNS) is controlled by active connection with other tissues with the support of CSF (Illes 2018). As CSF is derived from blood plasma and brain cells, it reflects both peripheral and CNS physiological activities (Reiber 2001). Though there is an important connection between blood and CSF, in non-mammalian vertebrates, little comparative work has been done to characterize these two significant fluids. Several studies have shown that adult invertebrate and vertebrate CNS experience neuronal birth and expansion, and that these cells can either replace or contribute to existing neural networks (Lindsey and Tropepe 2006). CSF proteins can regulate neurogenesis, brain homeostasis and participate in signalling during neuroinflammation. Even though birds represent valuable models for constitutive adult neurogenesis, current proteomic studies of the avian CSF are limited only to chicken embryos. In **Paper II,** for the very first time, we compared the domestic chicken to two parrot species: the budgerigar (*Melopsittacus undulatus*) and the cockatiel (*Nymphicus hollandicus*), which represent two distinct parrot clades (Psittacoidea and Cacatuoidea, respectively) (Provost, Joseph, and Smith 2018). We use liquid chromatography–tandem mass spectrometry (nLC-MS/MS) to explore the proteomic composition of CSF and plasma in adult chickens and parrots.

First, we revealed that the phylogenetically closest avian reference proteome has the highest efficacy in terms of protein identification success rates, even though a low proportion of proteins (7.2%, 18%, and 13.8% for chicken, budgerigar, and cockatiel, respectively) were mapped using other references. Following data filtering, we identified 483 proteins in the chicken CSF and PL, 641 proteins in the budgerigar, and 458 proteins in the cockatiel. We discovered more proteins in CSF than plasma, a finding that was consistent across all avian species in this study and is similar to what has previously been reported for humans in paired samples (Dayon et al. 2019; Guldbrandsen et al. 2014). In this study, we also studied the cockatiel, a species that currently lacks a genome sequence. We discovered that by using the sequence database from its closest evolutionary relative, the budgerigar, we could identify 458 proteins represented in the CSF and PL. This is more than what we obtained using other reference genomes (chicken, 327; zebra finch, 291). This pattern corresponded to our findings in other species included in this study. A similar approach has been used to describe the proteome of micro-organisms and frog *Xenopus laevis* microtubule-associated proteome (Wright, Beynon, and Hubbard 2010; Liska and Shevchenko 2003), but our study is the first one reporting a model of cross-species quantitative proteomics in distinct avian taxa. Comparative pathways analyse of CSF and blood plasma indicated clusters of proteins involved in neurogenesis, neural development, and neural differentiation overrepresented in CSF in each species similar to the study in rodents (Martin et al. 2009). Our study

provides the first insight into the proteomics of adult avian CSF and plasma and brings novel evidence supporting adult neurogenesis in birds. Understanding the molecular components involved in adult neurogenesis in birds, consistent with histological evidence, is critical for designing future experimental work in this system.

Brain inflammation is frequently linked with severe behavioural changes and health conditions like neurodegeneration (Kempuraj et al. 2017). Behavioural disorders have been identified in rodents and as well as cognitively advanced birds like parrots and zebra finch (Gaskins and Bergman 2011; Kinkaid et al. 2013; Ebisawa et al. 2021; Painsipp, Herzog, and Holzer 2010; Sulakhiya et al. 2016; White and Wright 2021). Symptoms like anxiety, over-eating, and self-damage (e.g.: feather plucking) are identified in parrots by veterinarians (van Zeeland et al. 2009; Péron and Grosset 2014; J. Chen et al. 2020), and zebra finches too showed altered, and repetitive behaviours (Tonna et al. 2020). Though we are not much aware of the possible causes of the behavioural disorders in these birds, in humans they are associated with neural inflammation (Swardfager et al. 2016). As we have the advantage of having the sequenced genome of zebra finch and budgerigar, genetically heterogeneous avian model species, we can adopt transcriptomic studies to understand the gene expression in the brain during peripheral inflammation.

RNA-sequencing is a fast-expanding technology, new methods are frequently developed. To assess these methods' relative performance, it is crucial to compare them with already-used techniques. In **Paper III**, we first compared the differential gene expression patterns in zebra finch skin during LPS-triggered peripheral inflammation obtained by using both the classical RNA-seq and the QuantSeq approaches. Previous research has shown that QuantSeq could be a viable alternative to traditional RNA-Seq in many applications, and it could be especially useful in studying the 3′UTR region of mRNA (Corley et al. 2019). Similar to previous study, the RNA-seq approach identified more differentially expressed genes (Corley et al. 2019) but failed to detect inflammatory markers. In our study, this could be because of the lower number of RNA-seq samples we used, in contrast to QuantSeq where we were able to sequence more samples due to its lower sequencing cost. Alternatively, QuantSeq results revealed more specific expression changes in genes regulating inflammation. Based on this result, we used QuantSeq to relate gene expression in the periphery and brain. Although we found only subtle differential gene expression changes in the brain during peripheral inflammation, several upregulated genes had clear immune function (e.g., *AVD-like* or *ACOD1*). The expression of Avidin (AVD)-related genes is increased in chickens during inflammation (Kunnas, Wallén, and Kulomaa 1993) as well as during bacterial and viral infections (Kunnas, Wallén, and Kulomaa 1993; Korpela et al. 1982). Higher expression of *AVD* can lead to the expression of stress proteins leading to the development of several neurodegenerative diseases, including Alzheimer's and Parkinson's (Guo et al. 2022). Similarly, *ACOD1* (aconitate decarboxylase 1, also known as immune responsive gene 1, *IRG1*) is a main regulator of immunometabolism during infection with significant anti-inflammatory properties (Wu 2022). Previous research in mice has shown that viral (Mills et al. 2018) and bacterial (Shi et al. 2005; Ganta et al. 2017) pathogens significantly increase *ACOD1* expression. For selected candidate target genes, QuantSeq results were validated with RT-qPCR. We show significant co-structure between peripheral and brain inflammation in our dataset. Our results obtained shows that zebra finches are able to regulate the peripheral inflammatory signals in brain. Our findings also suggest the advantages of the transcriptomic 3'-end approach for avian brain research in minimising the transcriptomic costs. It could be an alternative transcriptomic approach for classical RNA-seq if the research primarily involves gene expression studies.

The cannabinoids associated with the cannabinoid receptors (CNRs) are neuronal modulators interlinking the nervous and immune systems and have been indicated to have significant anti-

neuroinflammatory effects in humans (Klegeris, Bissonnette, and McGeer 2003; Domenici et al. 2006; Solas et al. 2013; Tao et al. 2016). Animals' immunological causes of behavioural problems are significantly less well understood than those in humans, and the interspecific variance in neuroimmune regulation networks is yet unclear. With their advanced cognitive capacities (Emery 2006), extensive neural networks (Olkowicz et al. 2016), and widespread psychopathologies (Kinkaid et al. 2013; Péron and Grosset 2014), parrots can aid in our understanding of the fundamental concepts behind how neuroinflammation affects behaviour. In our experiment with parrots, **Paper IV,** we show that the *CNR2* gene is pseudogenized in all parrots, revealing extensive karyotype rearrangements early in the parrot phylogeny as a cause of apparent *CNR2* pseudogenization (Nanda et al. 2007; Furo et al. 2018). We used genomic and transcriptomic approaches to confirm the gene loss. *CNR2* is the only gene consistently missing in parrots, in contrast to passerine birds (where it is present) (Maresz et al. 2005) making it a candidate for their susceptibility to neuroinflammation, according to our search for negative regulators of inflammatory responses. Our positive selection analysis suggested that the loss of *CNR2* in parrots did not result in compensatory adaptation in *CNR1*, and the comparative experimental results indicate that this event has an impact on neuroimmune control. Comparative study on zebra finches and parrot revealed that in contrast to the zebra finch, proinflammatory cytokines such as *IL1B* and *IL6* were upregulated in the hyperpallial tissue of CNR2-deficient budgerigars. This is further confirmed by the fact reported in other studies that parrots are particularly prone to bornavirus-related neuropathy (J. Chen et al. 2020; Rinder et al. 2009; Staeheli, Rinder, and Kaspers 2010; Rubbenstroth et al. 2012), and other parrot diseases such as bacteria, viruses, and fungi are suspected of causing behavioural abnormalities on a regular basis (Rubinstein and Lightfoot 2012; Speer 2014; Dovč et al. 2016). Thus, our findings imply that *CNR2* deficiency in parrots may affect control, dampening systemic proinflammatory signalling (for example, mediated by *IL1B* and *IL6*). CNR2-knock-out study on mice with significant immunopathology (Karmaus et al. 2013) supports our conclusion. Hence, our findings support the concept that *CNR2* deficiency has a regulatory role in neuroinflammation sensitivity, and they also suggest that parrots may be susceptible to neurological disorders. Our results provide significant insight into the evolutionary evidence for the functional impact of gene loss events during chromosomal rearrangements in addition to offering understanding to the variation in vulnerability to immunopathology between species. To better understand potential compensatory mechanisms in parrot immunity and their connections to the ecology and evolution of parrot infection, we stress the importance of further research like gene knock-out studies.

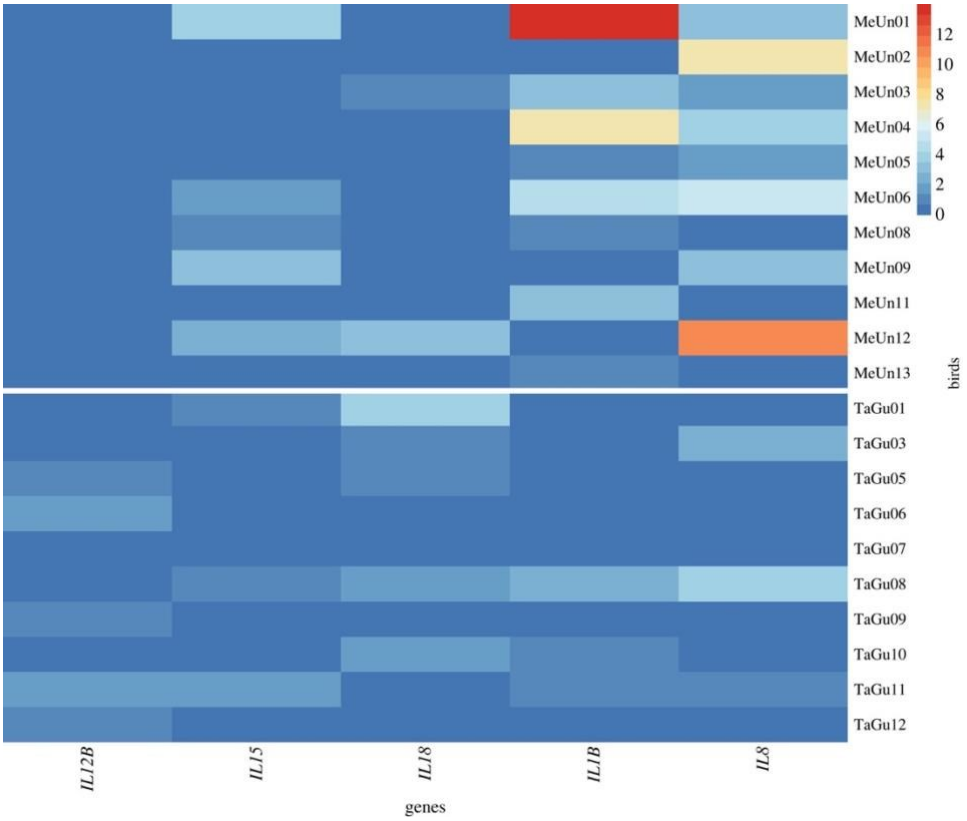

Figure 9: Heatmap showing differences in relative expression changes of proinflammatory cytokines in brains of LPS-stimulated budgerigars and zebra finches. MeUnX = budgerigar, X = number of the individual; TaGuY = zebra finch, Y = number of the individual. The heatmap colour ranges from blue (lowest upregulation in expression) to red (highest upregulation in expression). Courtesy (Divín et al. 2022) (License-CC BY-NC-SA 3.0)

Parrots and zebra finches are two of the most common pet birds. But understanding immune regulation in wild animals has been always challenging. Studies on their immune system and how it responds during the infection are crucial for the understanding of the evolution of the immune mechanisms and how they recover (Abolins et al. 2018). The primary aim of studying the immunology of the wild species is to link immune phenotype with host fitness in natural habitats (A. B. Pedersen and Babayan 2011). Discovery of new contagious diseases in wildlife especially those transmitted by the avian hosts have attracted world-wide interest over the past decades because of their high virulence and potential threat to domestic animals and humans (Staley and Bonneaud 2015; Benskin et al. 2009). These new disease emergences contributed to strong selection pressures on host immune responses by either resisting or tolerating the pathogen (Kerr 2012). Host-pathogen coevolution is a dynamic process that continuously contributes to the emergence of novel adaptations in both partners of this ecological interaction (Bonneaud et al. 2018). There are always costs in the activation of immune defences, either showing direct impacts like increased metabolic rate or as trade-offs with life processes like reduced growth and reproductive success (Brace et al. 2017; Hasselquist and Nilsson 2012). Better adaptation can optimise the level of the response and save the unnecessary costs. Understanding these shifts in regulating host immune responses to pathogens in wild birds, especially in non-model system is rare.

In collaboration with our US collaborators, our study aimed to investigate the evolutionary adaptations of gene expression in different house finch populations. These populations were infected with both the original (collected shortly after the disease onset) and evolved strains of MG (strains collected after 20

years of the disease outbreak). We utilized a unique research model system and employed 3' end transcriptomic approach to uncover these gene expression adaptations. Previous studies revealed that evolutionarily derived MG isolate cause increased periocular expression of pro-inflammatory cytokines resulting in increased levels of immunological activation and accompanying immunopathology, with potential direct benefits for MG transmission (Vinkler et al. 2018; Bale, Leon, and Hawley 2020). Birds with longer coevolutionary history with MG have prominent point tolerance than the ones in recent contact with MG (Adelman et al. 2013). In **Paper V** (manuscript)**,** we used conjunctival samples originating from a laboratory experiment performed in 2018 with four different house finch populations (differing in their coevolutionary history with MG) namely Virginia (VA), Iowa (IA), Arizona (AZ) and Hawaii (HI). These birds were infected with two different MG isolates (original VA1994 and evolved VA2013), where the Virginia birds have the longest coevolutionary history with MG and HI birds are naive to the pathogen. Thus, we had a unique possibility to investigate the coevolutionary changes in the host conjunctiva immunity and its dependence on increasing pathogen virulence.

We found significant alterations in gene expression during MG infection in the conjunctiva of house finches, we also found significant variance among the populations of these birds, which had a significant impact on the levels of overall conjunctival gene expression. Consistent with former studies (Adelman et al. 2013), our study revealed that the bird population with the longest coevolutionary history with MG (VA birds) show increased tolerance to MG, with up-regulated Th1 immunity (higher expression of *IL7* and *IL12B* in untreated controls compared to other population) compared to other three bird populations. The populations in recent contact with MG have an increased tendency for up-regulation of the *IL17* pathway (observed in AZ birds). Moreover, our findings show that infection with the more recent MG isolate (VA2013) causes higher levels of immune gene expression than infection with the original isolate (VA1994), a similar pattern was seen by (Vinkler et al. 2018). This affects not just the immune pathways, but also neurogenesis and neuron differentiation pathways. These effects can impact illness behaviour in birds, as observed in previous studies (Bouwman and Hawley 2010). Finally, we found *BCL10* as an important immune gene that changes its expression during the MG infection, varies in expression between individuals from different house finch populations, and changes its expression depending on the MG isolate infection. Though *BCL10* is an important adaptor molecule linking antigen receptor signalling cascades to NF-κB activation in lymphocytes (D. Wang et al. 2007), the precise role it plays in the immune regulation of house finches remains unclear.

General conclusions

In my doctoral thesis, I endeavored to describe the effects of inflammation on immune activity in both peripheral tissues and avian brains of wild and underrepresented avian models in neuro-immune studies at the transcriptomic and proteomic levels. I tried to improve the understanding on what are the ongoing adaptations of host immunity to effectively combat pathogens. This understanding is very crucial because infectious diseases have been constantly posing a threat to the health and well-being of all living organisms, and birds in particular play a significant role in spreading these diseases and act as reservoir hosts for emerging pathogens.

During my Ph.D. research tenure, I and my co-authors were able to describe the importance of studying evolutionary patterns of immune genes beyond MHCs and TLRs. Also was successful in explaining the significance of integrating other immunological data with evolutionary genetics using integrated analyses for the understanding of mechanisms involved in the evolution of host immune defense. This knowledge holds significant applications in wildlife conservation and mitigating the spread of infectious diseases. By comparing different transcriptomics approaches, we were able to show that 3' end sequencing (QuantSeq) could be an alternative method for classical RNA-seq for avian brain studies in a lower budget. Using this approach, in zebra finches and parrots, we showed how the immune system responds against peripheral inflammation and how it in turn affects the immune regulation in the brain. The findings highlight the neuro-immune changes occurring in the avian brain during an infection and how each bird modulates the immune regulation. We identified a previously unknown gene loss event in parrots that apparently made these birds more susceptible to neuroinflammation, compared to songbirds represented by zebra finches and eventually contributing to increased sensitivity to brain inflammation. Our findings offer vital insights not only into the variability in susceptibility to immunopathology between these species but also more generally into the functional effects of evolutionary gene loss events caused by chromosomal rearrangements. Our research confirms that these cognitively advanced birds are an excellent model for inflammation-related behavior studies. Additional research is necessary to elucidate possible compensatory mechanisms in parrot immunity, linking parrot infection ecology and evolution.

Despite recent research on wild vertebrates suggesting that tolerance to infection is a prevalent strategy used by hosts to minimize the fitness costs associated with encountering new pathogens, there is still a lack of evidence regarding the temporal dynamics of this evolutionary phenomenon and the specific immunological mechanisms responsible for the transition from resistance to tolerate in host-pathogen interactions. For the first time, our time dynamic gene expression study on conjunctival tissues of the wild host-pathogen model house finch-MG revealed the important immunological differences between house finch populations with different histories of MG endemism during the initial phase of the infection. We showed that birds from the Virginia population, the one with the longest co-evolutionary history with MG displayed increased tolerance to MG compared to other house finch populations. Our result demonstrates how the different population of the birds modulates the immune responses and maintains a balance between Th1 and Th17 pathway activation during the initial conjunctival response to different strains of MG infection in house finches. We also show how original and evolutionarily derived MG strains evoke different immune responses in the birds. The results also signify that evolutionarily derived pathogens triggered stronger immune responses compared to the original ones by affecting both immune and neurogenesis pathways. We strongly recommend further research in the brain of these house finch populations to identify the neuroinflammatory changes caused by the pathogens and how it affects the behavioral patterns of the birds. We should also investigate and validate gene expression using more specific techniques like qPCR and proteomics.

As a part of this research work, we also investigated a novel area of research by examining the CSF and plasma proteomes of adult birds for the first time. Most of the current proteomic studies of the avian CSF are limited to chicken embryos. To address this gap, using liquid chromatography-tandem mass spectrometry (nLC-MS/MS), we explored the proteomic composition of CSF and plasma in adult chickens and evolutionarily derived parrots. Our results indicate that proteins identified in abundance within the CSF were predominantly associated with functional pathways linked to neural function. These findings provide novel evidence supporting the presence of adult neurogenesis in birds, which correlates with the previous histological data study. As a next step, we recommend incorporating the molecular components involved in this process when designing and conducting future experiments.

Collectively, my thesis summarizes the integration of immunology, molecular biology, and evolutionary biology which interconnect animals' health with the environment and supports the One Health concept advocated by the World Health Organization (WHO). Immunological studies enabled a deeper understanding of immune regulations to pathogens, facilitating the identification of shared mechanisms between humans and animals, thereby enhancing our ability to combat zoonotic diseases. Gene level understanding of these immune modulations elucidated the genetic and molecular factors driving disease progression and host-pathogen interaction, providing valuable insights for disease surveillance, prevention, and control. Evolutionary biological aspects of our studies open the time dependent evolutionary disease dynamics in the immune regulatory mechanisms adopted by the evolving host and pathogen offering critical perspectives and the development of effective interventions. By harnessing the synergetic potential of these disciplines, we were able to better comprehend and address the challenges at the intersection of human, animal, and environmental health.

References

Able, Kenneth P, and James R Belthoff. 1998. "Rapid 'Evolution'of Migratory Behaviour in the Introduced House Finch of Eastern North America." *Proceedings of the Royal Society of London. Series B: Biological Sciences* 265 (1410): 2063–71.

Abolins, Stephen, Luke Lazarou, Laura Weldon, Louise Hughes, Elizabeth C. King, Paul Drescher, Michael J. O. Pocock, Julius C. R. Hafalla, Eleanor M. Riley, and Mark Viney. 2018. "The Ecology of Immune State in a Wild Mammal, Mus Musculus Domesticus." Edited by David Schneider. *PLOS Biology* 16 (4): e2003538. https://doi.org/10.1371/journal.pbio.2003538.

Acevedo-Whitehouse, Karina, and Andrew A. Cunningham. 2006. "Is MHC Enough for Understanding Wildlife Immunogenetics?" *Trends in Ecology & Evolution* 21 (8): 433–38. https://doi.org/10.1016/j.tree.2006.05.010.

Adelman, James S., Laila Kirkpatrick, Jessica L. Grodio, and Dana M. Hawley. 2013. "House Finch Populations Differ in Early Inflammatory Signaling and Pathogen Tolerance at the Peak of *Mycoplasma Gallisepticum* Infection." *The American Naturalist* 181 (5): 674–89. https://doi.org/10.1086/670024.

Alves, Joel M, Miguel Carneiro, Jade Y Cheng, Ana Lemos de Matos, Masmudur M Rahman, Liisa Loog, Paula F Campos, Nathan Wales, Anders Eriksson, and Andrea Manica. 2019. "Parallel Adaptation of Rabbit Populations to Myxoma Virus." *Science* 363 (6433): 1319–26.

Amarante-Mendes, Gustavo P., Sandy Adjemian, Laura Migliari Branco, Larissa C. Zanetti, Ricardo Weinlich, and Karina R. Bortoluci. 2018. "Pattern Recognition Receptors and the Host Cell Death Molecular Machinery." *Frontiers in Immunology* 9 (October): 2379. https://doi.org/10.3389/fimmu.2018.02379.

Andrews, Simon. 2010. "FastQC: A Quality Control Tool for High Throughput Sequence Data."

Bachy, Isabelle, Renata Kozyraki, and Marion Wassef. 2008. "The Particles of the Embryonic Cerebrospinal Fluid: How Could They Influence Brain Development?" *Brain Research Bulletin* 75 (2–4): 289–94.

Bale, Natalie M, Ariel E Leon, and Dana M Hawley. 2020. "Differential House Finch Leukocyte Profiles during Experimental Infection with Mycoplasma Gallisepticum Isolates of Varying Virulence." *Avian Pathology* 49 (4): 342–54.

Barbosa, Jomar M, Fernando Hiraldo, Miguel Á Romero, and José L Tella. 2021. "When Does Agriculture Enter into Conflict with Wildlife? A Global Assessment of Parrot–Agriculture Conflicts and Their Conservation Effects." *Diversity and Distributions* 27 (1): 4–17.

Barreiro, Luis B, and Lluís Quintana-Murci. 2010. "From Evolutionary Genetics to Human Immunology: How Selection Shapes Host Defence Genes." *Nature Reviews Genetics* 11 (1): 17–30.

Bateson, Melissa, and Gesa Feenders. 2010a. "The Use of Passerine Bird Species in Laboratory Research: Implications of Basic Biology for Husbandry and Welfare." *ILAR Journal* 51 (4): 394–408.

———. 2010b. "The Use of Passerine Bird Species in Laboratory Research: Implications of Basic Biology for Husbandry and Welfare." *ILAR Journal* 51 (4): 394–408.

Behrman, Emily L, Virginia M Howick, Martin Kapun, Fabian Staubach, Alan O Bergland, Dmitri A Petrov, Brian P Lazzaro, and Paul S Schmidt. 2018. "Rapid Seasonal Evolution in Innate Immunity of Wild Drosophila Melanogaster." *Proceedings of the Royal Society B: Biological Sciences* 285 (1870): 20172599.

Benskin, Clare McW. H., Kenneth Wilson, Keith Jones, and Ian R. Hartley. 2009. "Bacterial Pathogens in Wild Birds: A Review of the Frequency and Effects of Infection." *Biological Reviews* 84 (3): 349–73. https://doi.org/10.1111/j.1469-185X.2008.00076.x.

Bhat, Pooja, Thomas R Burkard, Veronika A Herzog, Andrea Pauli, and Stefan L Ameres. 2021. "Systematic Refinement of Gene Annotations by Parsing MRNA 3' End Sequencing Datasets." In *Methods in Enzymology*, 655:205–23. Elsevier.

Bloom, David E, Steven Black, and Rino Rappuoli. 2017. "Emerging Infectious Diseases: A Proactive Approach." *Proceedings of the National Academy of Sciences* 114 (16): 4055–59.

Bolger, Anthony M, Marc Lohse, and Bjoern Usadel. 2014. "Trimmomatic: A Flexible Trimmer for Illumina Sequence Data." *Bioinformatics* 30 (15): 2114–20.

Bonneaud, Camille, Mathieu Giraudeau, Luc Tardy, Molly Staley, Geoffrey E. Hill, and Kevin J. McGraw. 2018. "Rapid Antagonistic Coevolution in an Emerging Pathogen and Its Vertebrate Host." *Current Biology* 28 (18): 2978-2983.e5. https://doi.org/10.1016/j.cub.2018.07.003.

Borghans, José AM, Joost B Beltman, and Rob J De Boer. 2004. "MHC Polymorphism under Host-Pathogen Coevolution." *Immunogenetics* 55: 732–39.

Bouwman, Karen M, and Dana M Hawley. 2010. "Sickness Behaviour Acting as an Evolutionary Trap? Male House Finches Preferentially Feed near Diseased Conspecifics." *Biology Letters* 6 (4): 462–65.

Brace, Amber J., Marc J. Lajeunesse, Daniel R. Ardia, Dana M. Hawley, James S. Adelman, Katherine L. Buchanan, Jeanne M. Fair, Jennifer L. Grindstaff, Kevin D. Matson, and Lynn B. Martin. 2017. "Costs of Immune Responses Are Related to Host Body Size and Lifespan." *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* 327 (5): 254–61. https://doi.org/10.1002/jez.2084.

Brainard, Michael S., and Allison J. Doupe. 2002. "What Songbirds Teach Us about Learning." *Nature* 417 (6886): 351–58. https://doi.org/10.1038/417351a.

Buchmann, Kurt. 2014. "Evolution of Innate Immunity: Clues from Invertebrates via Fish to Mammals." *Frontiers in Immunology* 5: 459.

Buckingham, Lydia J, and Ben Ashby. 2022. "Coevolutionary Theory of Hosts and Parasites." *Journal of Evolutionary Biology* 35 (2): 205–24.

Cardinal, Pierre, Camille Monchaux de Oliveira, Julie Sauvant, Aline Foury, Muriel Darnaudéry, Sylvie Vancassel, Nathalie Castanon, and Lucile Capuron. 2021. "A New Experimental Design to Study Inflammation-Related versus Non-Inflammation-Related Depression in Mice." *Journal of Neuroinflammation* 18 (1): 290. https://doi.org/10.1186/s12974-021-02330-9.

Chan, David Tsz Chung, Emily Shui Kei Poon, Anson Tsz Chun Wong, and Simon Yung Wa Sin. 2021. "Global Trade in Parrots–Influential Factors of Trade and Implications for Conservation." *Global Ecology and Conservation* 30: e01784.

Chen, Jingshu, Jianhua Guo, Yanan Tian, and Ian Tizard. 2020. "The Pathogenesis of Proventricular Dilatation Disease Caused by Parrot Bornaviruses: A Possible Role for Neuropeptide Y (Npy)."

Chen, Linlin, Huidan Deng, Hengmin Cui, Jing Fang, Zhicai Zuo, Junliang Deng, Yinglun Li, Xun Wang, and Ling Zhao. 2018. "Inflammatory Responses and Inflammation-Associated Diseases in Organs." *Oncotarget* 9 (6): 7204–18. https://doi.org/10.18632/oncotarget.23208.

Corley, Susan M., Niamh M. Troy, Anthony Bosco, and Marc R. Wilkins. 2019. "QuantSeq. 3' Sequencing Combined with Salmon Provides a Fast, Reliable Approach for High Throughput RNA Expression Analysis." *Scientific Reports* 9 (1): 18895. https://doi.org/10.1038/s41598-019-55434-x.

Cox, Jürgen, Marco Y Hein, Christian A Luber, Igor Paron, Nagarjuna Nagaraj, and Matthias Mann. 2014. "Accurate Proteome-Wide Label-Free Quantification by Delayed Normalization and Maximal Peptide Ratio Extraction, Termed MaxLFQ." *Molecular & Cellular Proteomics* 13 (9): 2513–26.

Crystal, Jonathon D. 2012. "Prospective Cognition in Rats." *Learning and Motivation* 43 (4): 181–91.

Cunningham, Andrew A, Peter Daszak, and James LN Wood. 2017. "One Health, Emerging Infectious Diseases and Wildlife: Two Decades of Progress?" *Philosophical Transactions of the Royal Society B: Biological Sciences* 372 (1725): 20160167.

Cussen, Victoria A, and Joy A Mench. 2015. “The Relationship between Personality Dimensions and Resiliency to Environmental Stress in Orange-Winged Amazon Parrots (Amazona Amazonica), as Indicated by the Development of Abnormal Behaviors.” *PloS One* 10 (6): e0126170.

Dal Pont, Gabriela C., Bruna L. Belote, Annah Lee, Cristiano Bortoluzzi, Cinthia Eyng, Milena Sevastiyanova, Alireza Khadem, et al. 2021. “Novel Models for Chronic Intestinal Inflammation in Chickens: Intestinal Inflammation Pattern and Biomarkers.” *Frontiers in Immunology* 12 (May): 676628. https://doi.org/10.3389/fimmu.2021.676628.

Danilova, Nadia. 2006. “The Evolution of Immune Mechanisms.” *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 306 (6): 496–520.

Dayon, Loïc, Ornella Cominetti, Jérôme Wojcik, Antonio Núñez Galindo, Aikaterini Oikonomidi, Hugues Henry, Eugenia Migliavacca, Martin Kussmann, Gene L. Bowman, and Julius Popp. 2019. “Proteomes of Paired Human Cerebrospinal Fluid and Plasma: Relation to Blood–Brain Barrier Permeability in Older Adults.” *Journal of Proteome Research* 18 (3): 1162–74. https://doi.org/10.1021/acs.jproteome.8b00809.

Demas, Gregory, Randy Joe Nelson, and Randy Nelson. 2012. *Ecoimmunology*. OUP USA.

Dhondt, André A., Diane L. Tessaglia, and Roger L. Slothower. 1998. “EPIDEMIC MYCOPLASMAL CONJUNCTIVITIS IN HOUSE FINCHES FROM EASTERN NORTH AMERICA.” *Journal of Wildlife Diseases* 34 (2): 265–80. https://doi.org/10.7589/0090-3558-34.2.265.

Divín, Daniel, Mercedes Goméz Samblas, Nithya Kuttiyarthu Veetil, Eleni Voukali, Zuzana Świderská, Tereza Krajzingrová, Martin Těšický, et al. 2022. “Cannabinoid Receptor 2 Evolutionary Gene Loss Makes Parrots More Susceptible to Neuroinflammation.” *Proceedings of the Royal Society B: Biological Sciences* 289 (1988): 20221941. https://doi.org/10.1098/rspb.2022.1941.

Dobin, Alexander, Carrie A Davis, Felix Schlesinger, Jorg Drenkow, Chris Zaleski, Sonali Jha, Philippe Batut, Mark Chaisson, and Thomas R Gingeras. 2013. “STAR: Ultrafast Universal RNA-Seq Aligner.” *Bioinformatics* 29 (1): 15–21.

Domenici, Maria R, Shahnaz C Azad, Giovanni Marsicano, Anja Schierloh, Carsten T Wotjak, Hans-Ulrich Dodt, Walter Zieglgänsberger, Beat Lutz, and Gerhard Rammes. 2006. “Cannabinoid Receptor Type 1 Located on Presynaptic Terminals of Principal Neurons in the Forebrain Controls Glutamatergic Synaptic Transmission.” *Journal of Neuroscience* 26 (21): 5794–99.

Dong, Jianyi, Yuanyuan Chen, Fang Yang, Weidong Zhang, Kun Wei, Yongjian Xiong, Liang Wang, et al. 2021. “Naringin Exerts Therapeutic Effects on Mice Colitis: A Study Based on Transcriptomics Combined With Functional Experiments.” *Frontiers in Pharmacology* 12 (August): 729414. https://doi.org/10.3389/fphar.2021.729414.

Dovč, Alenka, Gregor Jereb, Uroš Krapež, Gordana Gregurić-Gračner, Štefan Pintarič, Brigita Slavec, Renata Lindtner Knific, Marjan Kastelic, Pavel Kvapil, and Jasna Mićunović. 2016. “Occurrence of Bacterial and Viral Pathogens in Common and Noninvasive Diagnostic Sampling from Parrots and Racing Pigeons in Slovenia.” *Avian Diseases* 60 (2): 487–92.

Ebisawa, Kazumasa, Shunya Nakayama, Chungyu Pai, Rie Kinoshita, and Hiroshi Koie. 2021. “Prevalence and Risk Factors for Feather-Damaging Behavior in Psittacine Birds: Analysis of a Japanese Nationwide Survey.” *PloS One* 16 (7): e0254610.

Ellenbroek, Bart, and Jiun Youn. 2016. “Rodent Models in Neuroscience Research: Is It a Rat Race?” *Disease Models & Mechanisms* 9 (10): 1079–87. https://doi.org/10.1242/dmm.026120.

Emery, Nathan J. 2006. “Cognitive Ornithology: The Evolution of Avian Intelligence.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 361 (1465): 23–43. https://doi.org/10.1098/rstb.2005.1736.

Ericson, Per GP, Seraina Klopfstein, Martin Irestedt, Jacqueline MT Nguyen, and Johan AA Nylander. 2014. “Dating the Diversification of the Major Lineages of Passeriformes (Aves).” *BMC Evolutionary Biology* 14: 1–15.

Fame, Ryann M, and Maria K Lehtinen. 2020. “Emergence and Developmental Roles of the Cerebrospinal Fluid System.” *Developmental Cell* 52 (3): 261–75.

Flores-Santin, Josele, and Warren W. Burggren. 2021. “Beyond the Chicken: Alternative Avian Models for Developmental Physiological Research.” *Frontiers in Physiology* 12 (October): 712633. https://doi.org/10.3389/fphys.2021.712633.

Forstmeier, Wolfgang, Gernot Segelbacher, Jakob C. Mueller, and Bart Kempenaers. 2007. “Genetic Variation and Differentiation in Captive and Wild Zebra Finches (*Taeniopygia Guttata*): ZEBRA FINCH POPULATION GENETICS.” *Molecular Ecology* 16 (19): 4039–50. https://doi.org/10.1111/j.1365-294X.2007.03444.x.

French, Chelsea E., Marites A. Sales, Samuel J. Rochell, Angeline Rodriguez, and Gisela F. Erf. 2020. “Local and Systemic Inflammatory Responses to Lipopolysaccharide in Broilers: New Insights Using a Two-Window Approach.” *Poultry Science* 99 (12): 6593–6605. https://doi.org/10.1016/j.psj.2020.09.078.

Furman, David, Judith Campisi, Eric Verdin, Pedro Carrera-Bastos, Sasha Targ, Claudio Franceschi, Luigi Ferrucci, et al. 2019. “Chronic Inflammation in the Etiology of Disease across the Life Span.” *Nature Medicine* 25 (12): 1822–32. https://doi.org/10.1038/s41591-019-0675-0.

Furo, Ivanete de Oliveira, Rafael Kretschmer, Patrícia CM O’Brien, Jorge C Pereira, Analía Del Valle Garnero, Ricardo J Gunski, Malcolm A Ferguson-Smith, and Edivaldo Herculano Corrêa De Oliveira. 2018. “Chromosome Painting in Neotropical Long-and Short-Tailed Parrots (Aves, Psittaciformes): Phylogeny and Proposal for a Putative Ancestral Karyotype for Tribe Arini.” *Genes* 9 (10): 491.

Ganta, Vijay Chaitanya, Min Hyub Choi, Anna Kutateladze, Todd E. Fox, Charles R. Farber, and Brian H. Annex. 2017. “A MicroRNA93–Interferon Regulatory Factor-9–Immunoresponsive Gene-1–Itaconic Acid Pathway Modulates M2-Like Macrophage Polarization to Revascularize Ischemic Muscle.” *Circulation* 135 (24): 2403–25. https://doi.org/10.1161/CIRCULATIONAHA.116.025490.

Gasch, Audrey P., Bret A. Payseur, and John E. Pool. 2016. “The Power of Natural Variation for Model Organism Biology.” *Trends in Genetics* 32 (3): 147–54. https://doi.org/10.1016/j.tig.2015.12.003.

Gaskins, Lori A, and Laurie Bergman. 2011. “Surveys of Avian Practitioners and Pet Owners Regarding Common Behavior Problems in Psittacine Birds.” *Journal of Avian Medicine and Surgery* 25 (2): 111–18.

Ge, Steven Xijin, Dongmin Jung, and Runan Yao. 2020. “ShinyGO: A Graphical Gene-Set Enrichment Tool for Animals and Plants.” Edited by Alfonso Valencia. *Bioinformatics* 36 (8): 2628–29. https://doi.org/10.1093/bioinformatics/btz931.

Griffith, Simon C, Riccardo Ton, Laura L Hurley, Callum S McDiarmid, and Hector Pacheco-Fuentes. 2021. “The Ecology of the Zebra Finch Makes It a Great Laboratory Model but an Outlier amongst Passerine Birds.” *Birds* 2 (1): 60–76.

Guldbrandsen, Astrid, Heidrun Vethe, Yehia Farag, Eystein Oveland, Hilde Garberg, Magnus Berle, Kjell-Morten Myhr, Jill A Opsahl, Harald Barsnes, and Frode S Berven. 2014. “In-Depth Characterization of the Cerebrospinal Fluid (CSF) Proteome Displayed through the CSF Proteome Resource (CSF-PR).” *Molecular & Cellular Proteomics* 13 (11): 3152–63.

Guo, Qingyun, Ichiro Kawahata, An Cheng, Wenbin Jia, Haoyang Wang, and Kohji Fukunaga. 2022. “Fatty Acid-Binding Proteins: Their Roles in Ischemic Stroke and Potential as Drug Targets.” *International Journal of Molecular Sciences* 23 (17): 9648.

Hains, Taylor, Kathleen O’Neill, Jafet Velez, Nancy Speed, Susan Clubb, Taras Oleksyk, and Stacy Pirro. 2020. “The Complete Genome Sequences of 22 Parrot Species (Psittaciformes, Aves).” https://doi.org/10.12688/f1000research.25560.1.

Hasselquist, Dennis, and Jan-Åke Nilsson. 2012. “Physiological Mechanisms Mediating Costs of Immune Responses: What Can We Learn from Studies of Birds?” *Animal Behaviour* 83 (6): 1303–12. https://doi.org/10.1016/j.anbehav.2012.03.025.

Hawley, Dana M, Erik E Osnas, Andrew P Dobson, Wesley M Hochachka, David H Ley, and Andre A Dhondt. 2013a. “Parallel Patterns of Increased Virulence in a Recently Emerged Wildlife Pathogen.” *PLOS Biology* 11 (5): 11.

Hawley, Dana M, Erik E Osnas, Andrew P Dobson, Wesley M Hochachka, David H Ley, and André A Dhondt. 2013b. “Parallel Patterns of Increased Virulence in a Recently Emerged Wildlife Pathogen.” *PLoS Biology* 11 (5): e1001570.

Hess, Laurie, Tracy Bartick, and Heidi Hoefer. 1998. “Clostridium Tertium Infection in a Moluccan Cockatoo (Cacatua Moluccensis) with Megacolon.” *Journal of Avian Medicine and Surgery*, 30–35.

Hickman, DL, J Johnson, TH Vemulapalli, JR Crisler, and R Shepherd. 2017. “Commonly Used Animal Models.” *Principles of Animal Research for Graduate and Undergraduate Students*, 117.

Hill, Geoffrey E, and Kristy L Farmer. 2005. “Carotenoid-Based Plumage Coloration Predicts Resistance to a Novel Parasite in the House Finch.” *Naturwissenschaften* 92: 30–34.

Hochachka, Wesley M, and Andre A Dhondt. n.d. “Density-Dependent Decline of Host Abundance Resulting from a New Infectious Disease,” 4.

Holt, Robert A. 2015. “Immunogenomics: A Foundation for Intelligent Immune Design.” *Genome Medicine* 7 (1): 1–3.

Howe, Kevin L, Premanand Achuthan, James Allen, Jamie Allen, Jorge Alvarez-Jarreta, M Ridwan Amode, Irina M Armean, et al. 2021. “Ensembl 2021.” *Nucleic Acids Research* 49 (D1): D884–91. https://doi.org/10.1093/nar/gkaa942.

Illes, Sebastian. 2018. “Chapter 3 - More than a Drainage Fluid: The Role of CSF in Signaling in the Brain and Other Effects on Brain Tissue.” In *Handbook of Clinical Neurology*, edited by Florian Deisenhammer, Charlotte E. Teunissen, and Hayrettin Tumani, 146:33–46. Elsevier. https://doi.org/10.1016/B978-0-12-804279-3.00003-4.

Jarvis, Seth, Nicol Birsa, Maria Secrier, Pietro Fratta, and Vincent Plagnol. 2020. “A Comparison of Low Read Depth QuantSeq 3’ Sequencing to Total RNA-Seq in FUS Mutant Mice.” *Frontiers in Genetics* 11: 1412. https://doi.org/10.3389/fgene.2020.562445.

Karin, Michael, Toby Lawrence, and Victor Nizet. 2006. “Innate Immunity Gone Awry: Linking Microbial Infections to Chronic Inflammation and Cancer.” *Cell* 124 (4): 823–35. https://doi.org/10.1016/j.cell.2006.02.016.

Karmaus, Peer WF, Weimin Chen, Robert Crawford, Barbara LF Kaplan, and Norbert E Kaminski. 2013. “Δ9-Tetrahydrocannabinol Impairs the Inflammatory Response to Influenza Infection: Role of Antigen-Presenting Cells and the Cannabinoid Receptors 1 and 2.” *Toxicological Sciences* 131 (2): 419–33.

Kaufman, Jim. 2010. “Evolution and Immunity.” *Immunology* 130 (4): 459–62.

Kawai, T., and S. Akira. 2006. “TLR Signaling.” *Cell Death & Differentiation* 13 (5): 816–25. https://doi.org/10.1038/sj.cdd.4401850.

Kempuraj, Duraisamy, Ramasamy Thangavel, Govindhasamy P. Selvakumar, Smita Zaheer, Mohammad E. Ahmed, Sudhanshu P. Raikwar, Haris Zahoor, et al. 2017. “Brain and Peripheral Atypical Inflammatory Mediators Potentiate Neuroinflammation and Neurodegeneration.” *Frontiers in Cellular Neuroscience* 11 (July): 216. https://doi.org/10.3389/fncel.2017.00216.

Kerr, Peter J. 2012. “Myxomatosis in Australia and Europe: A Model for Emerging Infectious Diseases.” *Antiviral Research* 93 (3): 387–415. https://doi.org/10.1016/j.antiviral.2012.01.009.

Khan, Imran, Emanuel Maldonado, Liliana Silva, Daniela Almeida, Warren E Johnson, Stephen J O’Brien, Guojie Zhang, Erich D Jarvis, M Thomas P Gilbert, and Agostinho Antunes. 2019. “The Vertebrate TLR Supergene Family Evolved Dynamically by Gene Gain/Loss and Positive Selection Revealing a Host–Pathogen Arms Race in Birds.” *Diversity* 11 (8): 131.

Kinkaid, Heather Y McDonald, Daniel S Mills, Steve G Nichols, Rebecca K Meagher, and Georgia J Mason. 2013. “Feather-Damaging Behaviour in Companion Parrots: An Initial Analysis of Potential Demographic Risk Factors.” *Avian Biology Research* 6 (4): 289–96.

Klegeris, Andis, Christopher J Bissonnette, and Patrick L McGeer. 2003. “Reduction of Human Monocytic Cell Neurotoxicity and Cytokine Secretion by Ligands of the Cannabinoid-type CB2 Receptor.” *British Journal of Pharmacology* 139 (4): 775–86.

Korpela, Jukka, Markku Kulomaa, Pentti Tuohimaa, and Antti Vaheri. 1982. “Induction of Avidin in Chickens Infected with the Acute Leukemia Virus OK 10.” *International Journal of Cancer* 30 (4): 461–64.

Kunnas, Tarja A., Mika J. Wallén, and Markku S. Kulomaa. 1993. “Induction of Chicken Avidin and Related MRNAs after Bacterial Infection.” *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression* 1216 (3): 441–45. https://doi.org/10.1016/0167-4781(93)90012-3.

Land, Walter Gottlieb. 2020. “Pattern Recognition Molecules.” In *Damage-Associated Molecular Patterns in Human Diseases*, by Walter Gottlieb Land, 13–65. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-53868-2_2.

Langwig, Kate E, Joseph R Hoyt, Katy L Parise, Winifred F Frick, Jeffrey T Foster, and A Marm Kilpatrick. 2017. “Resistance in Persisting Bat Populations after White-Nose Syndrome Invasion.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 372 (1712): 20160044.

Lehtinen, Maria K, Mauro W Zappaterra, Xi Chen, Yawei J Yang, Anthony D Hill, Melody Lun, Thomas Maynard, Dilenny Gonzalez, Seonhee Kim, and Ping Ye. 2011. “The Cerebrospinal Fluid Provides a Proliferative Niche for Neural Progenitor Cells.” *Neuron* 69 (5): 893–905.

Ley, David H., J. Edward Berkhoff, and Judith M. McLaren. 1996. “Mycoplasma Gallisepticum Isolated from House Finches (Carpodacus Mexicanus) with Conjunctivitis.” *Avian Diseases* 40 (2): 480. https://doi.org/10.2307/1592250.

Liao, Yang, Gordon K. Smyth, and Wei Shi. 2014. “FeatureCounts: An Efficient General Purpose Program for Assigning Sequence Reads to Genomic Features.” *Bioinformatics* 30 (7): 923–30. https://doi.org/10.1093/bioinformatics/btt656.

Lindsey, Benjamin W, and Vincent Tropepe. 2006. “A Comparative Framework for Understanding the Biological Principles of Adult Neurogenesis.” *Progress in Neurobiology* 80 (6): 281–307.

Liska, Adam J., and Andrej Shevchenko. 2003. “Expanding the Organismal Scope of Proteomics: Cross-Species Protein Identification by Mass Spectrometry and Its Implications.” *PROTEOMICS* 3 (1): 19–28. https://doi.org/10.1002/pmic.200390004.

Ma, Feiyang, Brie K. Fuqua, Yehudit Hasin, Clara Yukhtman, Chris D. Vulpe, Aldons J. Lusis, and Matteo Pellegrini. 2019. “A Comparison between Whole Transcript and 3’ RNA Sequencing Methods Using Kapa and Lexogen Library Preparation Methods.” *BMC Genomics* 20 (1): 9. https://doi.org/10.1186/s12864-018-5393-3.

Magalhães, João Pedro. 2015. “The Big, the Bad and the Ugly: Extreme Animals as Inspiration for Biomedical Research.” *EMBO Reports* 16 (7): 771–76. https://doi.org/10.15252/embr.201540606.

Maresz, Katarzyna, Erica J Carrier, Eugene D Ponomarev, Cecilia J Hillard, and Bonnie N Dittel. 2005. “Modulation of the Cannabinoid CB2 Receptor in Microglial Cells in Response to Inflammatory Stimuli.” *Journal of Neurochemistry* 95 (2): 437–45.

Martin, C., M.I. Alonso, C. Santiago, J.A. Moro, A. De la Mano, R. Carretero, and A. Gato. 2009. “Early Embryonic Brain Development in Rats Requires the Trophic Influence of Cerebrospinal Fluid.” *International Journal of Developmental Neuroscience* 27 (7): 733–40. https://doi.org/10.1016/j.ijdevneu.2009.06.002.

McTaggart, Seanna J, Darren J Obbard, Claire Conlon, and Tom J Little. 2012. “Immune Genes Undergo More Adaptive Evolution than Non-Immune System Genes in Daphnia Pulex.” *BMC Evolutionary Biology* 12 (1): 1–11.

Medina-García, Angela, Jodie M Jawor, and Timothy F Wright. 2017. “Cognition, Personality, and Stress in Budgerigars, Melopsittacus Undulatus.” Edited by Jonathan Pruitt. *Behavioral Ecology* 28 (6): 1504–16. https://doi.org/10.1093/beheco/arx116.

Medzhitov, Ruslan, Paula Preston-Hurlburt, and Charles A. Janeway. 1997. “A Human Homologue of the Drosophila Toll Protein Signals Activation of Adaptive Immunity.” *Nature* 388 (6640): 394–97. https://doi.org/10.1038/41131.

Mering, Christian von, Martijn Huynen, Daniel Jaeggi, Steffen Schmidt, Peer Bork, and Berend Snel. 2003. “STRING: A Database of Predicted Functional Associations between Proteins.” *Nucleic Acids Research* 31 (1): 258–61.

Messa Júnior, Augusto, Paula Taunde, Ana Felicidade Zandamela, Alberto Pondja Junior, Abel Chilundo, Rosa Costa, and Custódio Gabriel Bila. 2017. “Serological Screening Suggests Extensive Presence of *Mycoplasma Gallisepticum* and *Mycoplasma Synoviae* in Backyard Chickens in Southern Mozambique.” *Journal of Veterinary Medicine* 2017 (January): 1–4. https://doi.org/10.1155/2017/2743187.

Mestas, Javier, and Christopher CW Hughes. 2004. “Of Mice and Not Men: Differences between Mouse and Human Immunology.” *The Journal of Immunology* 172 (5): 2731–38.

Mills, Evanna L., Dylan G. Ryan, Hiran A. Prag, Dina Dikovskaya, Deepthi Menon, Zbigniew Zaslona, Mark P. Jedrychowski, et al. 2018. “Itaconate Is an Anti-Inflammatory Metabolite That Activates Nrf2 via Alkylation of KEAP1.” *Nature* 556 (7699): 113–17. https://doi.org/10.1038/nature25986.

Moll, Pamela, Michael Ante, Alexander Seitz, and Torsten Reda. 2014. “QuantSeq 3′ MRNA Sequencing for RNA Quantification.” *Nature Methods* 11 (12): i–iii. https://doi.org/10.1038/nmeth.f.376.

Morimoto, Keiko, and Kazunori Nakajima. 2019. “Role of the Immune System in the Development of the Central Nervous System.” *Frontiers in Neuroscience* 13: 916.

Nanda, I, E Karl, Darren K Griffin, M Schartl, and Michael Schmid. 2007. “Chromosome Repatterning in Three Representative Parrots (Psittaciformes) Inferred from Comparative Chromosome Painting.” *Cytogenetic and Genome Research* 117 (1–4): 43–53.

Nathan, Carl, and Aihao Ding. 2010. “Nonresolving Inflammation.” *Cell* 140 (6): 871–82.

Newton, Kim, and Vishva M Dixit. 2012. “Signaling in Innate Immunity and Inflammation.” *Cold Spring Harbor Perspectives in Biology* 4 (3): a006049.

Ohmer, Michel EB, David Costantini, Gábor Á Czirják, Cynthia J Downs, Laura V Ferguson, Andy Flies, Craig E Franklin, Ahab N Kayigwe, Sarah Knutie, and Corinne L Richards-Zawacki. 2021. “Applied Ecoimmunology: Using Immunological Tools to Improve Conservation Efforts in a Changing World.” *Conservation Physiology* 9 (1): coab074.

Oksche, A, Donald S Farner, DL Serventy, Frank Wolff, and C_A Nicholls. 1963. “The Hypothalamo-Hypophysial Neuro Secretory System of the Zebra Finch, Taeniopygia Castanotis.” *Zeitschrift Für Zellforschung Und Mikroskopische Anatomie* 58 (6): 846–914.

Olkowicz, Seweryn, Martin Kocourek, Radek K. Lučan, Michal Porteš, W. Tecumseh Fitch, Suzana Herculano-Houzel, and Pavel Němec. 2016. “Birds Have Primate-like Numbers of Neurons in the Forebrain.” *Proceedings of the National Academy of Sciences* 113 (26): 7255–60. https://doi.org/10.1073/pnas.1517131113.

Owen-Ashley, Noah T., and John C. Wingfield. 2007. “Acute Phase Responses of Passerine Birds: Characterization and Seasonal Variation.” *Journal of Ornithology* 148 (S2): 583–91. https://doi.org/10.1007/s10336-007-0197-2.

Painsipp, Evelin, Herbert Herzog, and Peter Holzer. 2010. “Evidence from Knockout Mice That Neuropeptide-Y Y2 and Y4 Receptor Signalling Prevents Long-Term Depression-like Behaviour Caused by Immune Challenge.” *Journal of Psychopharmacology* 24 (10): 1551–60. https://doi.org/10.1177/0269881109348171.

Papadopoulos, George, Carolyn D. Kramer, Connie S. Slocum, Ellen O. Weinberg, Ning Hua, Cynthia V. Gudino, James A. Hamilton, and Caroline A. Genco. 2014. “A Mouse Model for Pathogen-

Induced Chronic Inflammation at Local and Systemic Sites." *Journal of Visualized Experiments*, no. 90 (August): 51556. https://doi.org/10.3791/51556.

Papkou, Andrei, Thiago Guzella, Wentao Yang, Svenja Koepper, Barbara Pees, Rebecca Schalkowski, Mike-Christoph Barg, Philip C. Rosenstiel, Henrique Teotónio, and Hinrich Schulenburg. 2019. "The Genomic Basis of Red Queen Dynamics during Rapid Reciprocal Host–Pathogen Coevolution." *Proceedings of the National Academy of Sciences* 116 (3): 923–28. https://doi.org/10.1073/pnas.1810402116.

Pavlovich, Stephanie S, Sean P Lovett, Galina Koroleva, Jonathan C Guito, Catherine E Arnold, Elyse R Nagle, Kirsten Kulcsar, Albert Lee, Françoise Thibaud-Nissen, and Adam J Hume. 2018. "The Egyptian Rousette Genome Reveals Unexpected Features of Bat Antiviral Immunity." *Cell* 173 (5): 1098–1110.

Pedersen, Alyssa L, Lars H Nelson, and Colin J Saldanha. 2016. "Centrally Synthesized Estradiol Is a Potent Anti-Inflammatory in the Injured Zebra Finch Brain." *Endocrinology* 157 (5): 2041–51.

Pedersen, Amy B., and Simon A. Babayan. 2011. "Wild Immunology: WILD IMMUNOLOGY." *Molecular Ecology* 20 (5): 872–80. https://doi.org/10.1111/j.1365-294X.2010.04938.x.

Péron, F., and C. Grosset. 2014. "The Diet of Adult Psittacids: Veterinarian and Ethological Approaches." *Journal of Animal Physiology and Animal Nutrition* 98 (3): 403–16. https://doi.org/10.1111/jpn.12103.

Poulose, Shibu M, Marshall G Miller, Tammy Scott, and Barbara Shukitt-Hale. 2017. "Nutritional Factors Affecting Adult Neurogenesis and Cognitive Function." *Advances in Nutrition* 8 (6): 804–11.

Provost, Kaiya L, Leo Joseph, and Brian Tilston Smith. 2018. "Resolving a Phylogenetic Hypothesis for Parrots: Implications from Systematics to Conservation." *Emu-Austral Ornithology* 118 (1): 7–21.

Raudvere, Uku, Liis Kolberg, Ivan Kuzmin, Tambet Arak, Priit Adler, Hedi Peterson, and Jaak Vilo. 2019. "G:Profiler: A Web Server for Functional Enrichment Analysis and Conversions of Gene Lists (2019 Update)." *Nucleic Acids Research* 47 (W1): W191–98. https://doi.org/10.1093/nar/gkz369.

Ravi, Meghna, Andrew H Miller, and Vasiliki Michopoulos. 2021. "The Immunology of Stress and the Impact of Inflammation on the Brain and Behaviour." *BJPsych Advances* 27 (3): 158–65.

Reiber, Hansotto. 2001. "Dynamics of Brain-Derived Proteins in Cerebrospinal Fluid." *Clinica Chimica Acta* 310 (2): 173–86.

Rinder, Monika, Andreas Ackermann, Hermann Kempf, Bernd Kaspers, Rüdiger Korbel, and Peter Staeheli. 2009. "Broad Tissue and Cell Tropism of Avian Bornavirus in Parrots with Proventricular Dilatation Disease." *Journal of Virology* 83 (11): 5401–7.

Rubbenstroth, D, M Rinder, B Kaspers, and P Staeheli. 2012. "Efficient Isolation of Avian Bornaviruses (ABV) from Naturally Infected Psittacine Birds and Identification of a New ABV Genotype from a Salmon-Crested Cockatoo (Cacatua Moluccensis)." *Veterinary Microbiology* 161 (1–2): 36–42.

Rubinstein, Jonathan, and Teresa Lightfoot. 2012. "Feather Loss and Feather Destructive Behavior in Pet Birds." *Journal of Exotic Pet Medicine* 21 (3): 219–34.

Sawamoto, Kazunobu, Hynek Wichterle, Oscar Gonzalez-Perez, Jeremy A Cholfin, Masayuki Yamada, Nathalie Spassky, Noel S Murcia, Jose Manuel Garcia-Verdugo, Oscar Marin, and John LR Rubenstein. 2006. "New Neurons Follow the Flow of Cerebrospinal Fluid in the Adult Brain." *Science* 311 (5761): 629–32.

Shi, Shuangping, Antje Blumenthal, Christopher M. Hickey, Sheetal Gandotra, David Levy, and Sabine Ehrt. 2005. "Expression of Many Immunologically Important Genes in Mycobacterium Tuberculosis-Infected Macrophages Is Independent of Both TLR2 and TLR4 but Dependent on IFN-Aβ Receptor and STAT11." *The Journal of Immunology* 175 (5): 3318–28. https://doi.org/10.4049/jimmunol.175.5.3318.

Sironi, Manuela, Rachele Cagliani, Diego Forni, and Mario Clerici. 2015. “Evolutionary Insights into Host–Pathogen Interactions from Mammalian Sequence Data.” *Nature Reviews Genetics* 16 (4): 224–36. https://doi.org/10.1038/nrg3905.

Smedley, Damian, Syed Haider, Steffen Durinck, Luca Pandini, Paolo Provero, James Allen, Olivier Arnaiz, et al. 2015. “The BioMart Community Portal: An Innovative Alternative to Large, Centralized Data Repositories.” *Nucleic Acids Research* 43 (W1): W589–98. https://doi.org/10.1093/nar/gkv350.

Solas, Maite, Paul T Francis, Rafael Franco, and Maria J Ramirez. 2013. “CB2 Receptor and Amyloid Pathology in Frontal Cortex of Alzheimer’s Disease Patients.” *Neurobiology of Aging* 34 (3): 805–8.

Speer, Brian. 2014. “Normal and Abnormal Parrot Behavior.” *Journal of Exotic Pet Medicine* 23 (3): 230–33.

Spierings, Michelle J, and Carel Ten Cate. 2016. “Zebra Finches as a Model Species to Understand the Roots of Rhythm.” *Frontiers in Neuroscience* 10: 345.

Staeheli, Peter, Monika Rinder, and Bernd Kaspers. 2010. “Avian Bornavirus Associated with Fatal Disease in Psittacine Birds.” *Journal of Virology* 84 (13): 6269–75.

Staley, M., and C. Bonneaud. 2015. “Immune Responses of Wild Birds to Emerging Infectious Diseases.” *Parasite Immunology* 37 (5): 242–54. https://doi.org/10.1111/pim.12191.

Stapley, Jessica, Tim R Birkhead, Terry Burke, and Jon Slate. 2008. “A Linkage Map of the Zebra Finch Taeniopygia Guttata Provides New Insights into Avian Genome Evolution.” *Genetics* 179 (1): 651–67.

Straub, Rainer H. 2017. “The Brain and Immune System Prompt Energy Shortage in Chronic Inflammation and Ageing.” *Nature Reviews Rheumatology* 13 (12): 743–51.

Straub, RH, M Cutolo, F Buttgereit, and G Pongratz. 2010. “Energy Regulation and Neuroendocrine–Immune Control in Chronic Inflammatory Diseases.” *Journal of Internal Medicine* 267 (6): 543–60.

Subramanian, Aravind, Pablo Tamayo, Vamsi K Mootha, Sayan Mukherjee, Benjamin L Ebert, Michael A Gillette, Amanda Paulovich, Scott L Pomeroy, Todd R Golub, and Eric S Lander. 2005. “Gene Set Enrichment Analysis: A Knowledge-Based Approach for Interpreting Genome-Wide Expression Profiles.” *Proceedings of the National Academy of Sciences* 102 (43): 15545–50.

Suh, Alexander, Martin Paus, Martin Kiefmann, Gennady Churakov, Franziska Anni Franke, Jürgen Brosius, Jan Ole Kriegs, and Jürgen Schmitz. 2011. “Mesozoic Retroposons Reveal Parrots as the Closest Living Relatives of Passerine Birds.” *Nature Communications* 2 (1): 443.

Sulakhiya, Kunjbihari, Gohil Pratik Keshavlal, Babul B. Bezbaruah, Shubham Dwivedi, Satendra Singh Gurjar, Nitin Munde, Ashok Jangra, Mangala Lahkar, and Ranadeep Gogoi. 2016. “Lipopolysaccharide Induced Anxiety- and Depressive-like Behaviour in Mice Are Prevented by Chronic Pre-Treatment of Esculetin.” *Neuroscience Letters* 611 (January): 106–11. https://doi.org/10.1016/j.neulet.2015.11.031.

Sung, Pi-Shan, Po-Yu Lin, Chi-Hung Liu, Hui-Chen Su, and Kuen-Jer Tsai. 2020. “Neuroinflammation and Neurogenesis in Alzheimer’s Disease and Potential Therapeutic Approaches.” *International Journal of Molecular Sciences* 21 (3): 701.

Swardfager, Walter, Joshua D Rosenblat, Meriem Benlamri, and Roger S McIntyre. 2016. “Mapping Inflammation onto Mood: Inflammatory Mediators of Anhedonia.” *Neuroscience & Biobehavioral Reviews* 64: 148–66.

Świderská, Zuzana, Adéla Šmídová, Lucie Buchtová, Anna Bryjová, Anežka Fabiánová, Pavel Munclinger, and Michal Vinkler. 2018. “Avian Toll-like Receptor Allelic Diversity Far Exceeds Human Polymorphism: An Insight from Domestic Chicken Breeds.” *Scientific Reports* 8 (1): 17878.

Takeuchi, Osamu, and Shizuo Akira. 2010. “Pattern Recognition Receptors and Inflammation.” *Cell* 140 (6): 805–20. https://doi.org/10.1016/j.cell.2010.01.022.

Tao, Yihao, Lin Li, Bing Jiang, Zhou Feng, Liming Yang, Jun Tang, Qianwei Chen, Jianbo Zhang, Qiang Tan, and Hua Feng. 2016. “Cannabinoid Receptor-2 Stimulation Suppresses Neuroinflammation by Regulating Microglial M1/M2 Polarization through the CAMP/PKA Pathway in an Experimental GMH Rat Model.” *Brain, Behavior, and Immunity* 58: 118–29.

Tavares, Erika Sendra, Allan J Baker, Sérgio Luiz Pereira, and Cristina Yumi Miyaki. 2006. “Phylogenetic Relationships and Historical Biogeography of Neotropical Parrots (Psittaciformes: Psittacidae: Arini) Inferred from Mitochondrial and Nuclear DNA Sequences.” *Systematic Biology* 55 (3): 454–70. https://doi.org/10.1080/10635150600697390.

Tella, José L., Guillermo Blanco, and Martina Carrete. 2022. “Recent Advances in Parrot Research and Conservation.” *Diversity* 14 (6). https://doi.org/10.3390/d14060419.

Těšický, Martin, Hana Velová, Marian Novotný, Jakub Kreisinger, Vladimír Beneš, and Michal Vinkler. 2020. “Positive Selection and Convergent Evolution Shape Molecular Phenotypic Traits of Innate Immunity Receptors in Tits (Paridae).” *Molecular Ecology* 29 (16): 3056–70.

Těšický, Martin, and Michal Vinkler. 2015. “Trans-Species Polymorphism in Immune Genes: General Pattern or MHC-Restricted Phenomenon?” *Journal of Immunology Research* 2015.

The UniProt Consortium, Alex Bateman, Maria-Jesus Martin, Sandra Orchard, Michele Magrane, Rahat Agivetova, Shadab Ahmad, et al. 2021. “UniProt: The Universal Protein Knowledgebase in 2021.” *Nucleic Acids Research* 49 (D1): D480–89. https://doi.org/10.1093/nar/gkaa1100.

Thomma, Bart P.H.J., Thorsten Nürnberger, and Matthieu H.A.J. Joosten. 2011. “Of PAMPs and Effectors: The Blurred PTI-ETI Dichotomy.” *The Plant Cell* 23 (1): 4–15. https://doi.org/10.1105/tpc.110.082602.

Tonna, Matteo, Davide Ponzi, Paola Palanza, Carlo Marchesi, and Stefano Parmigiani. 2020. “Proximate and Ultimate Causes of Ritual Behavior.” *Behavioural Brain Research* 393: 112772.

Träger, Ulrike, and Sarah J. Tabrizi. 2013. “Peripheral Inflammation in Neurodegeneration.” *Journal of Molecular Medicine* 91 (6): 673–81. https://doi.org/10.1007/s00109-013-1026-0.

Vénéreau, Emilie, Chiara Ceriotti, and Marco Emilio Bianchi. 2015. “DAMPs from Cell Death to New Life.” *Frontiers in Immunology* 6 (August). https://doi.org/10.3389/fimmu.2015.00422.

Vergara-Tabares, David L, Javier M Cordier, Marcos A Landi, George Olah, and Javier Nori. 2020. “Global Trends of Habitat Destruction and Consequences for Parrot Conservation.” *Global Change Biology* 26 (8): 4251–62.

Villeda, Saul A, Jian Luo, Kira I Mosher, Bende Zou, Markus Britschgi, Gregor Bieri, Trisha M Stan, Nina Fainberg, Zhaoqing Ding, and Alexander Eggel. 2011. “The Ageing Systemic Milieu Negatively Regulates Neurogenesis and Cognitive Function.” *Nature* 477 (7362): 90–94.

Vinkler, Michal, Steven R Fiddaman, Martin Těšický, Emily A O’Connor, Anna E Savage, Tobias L Lenz, Adrian L Smith, Jim Kaufman, Daniel I Bolnick, and Charli S Davies. 2023. “Understanding the Evolution of Immune Genes in Jawed Vertebrates.” *Journal of Evolutionary Biology*.

Vinkler, Michal, Ariel E. Leon, Laila Kirkpatrick, Rami A. Dalloul, and Dana M. Hawley. 2018. “Differing House Finch Cytokine Expression Responses to Original and Evolved Isolates of Mycoplasma Gallisepticum.” *Frontiers in Immunology* 9 (January): 13. https://doi.org/10.3389/fimmu.2018.00013.

Vo, Thu Thi Minh, Tuan Viet Nguyen, Gianluca Amoroso, Tomer Ventura, and Abigail Elizur. 2021. “Deploying New Generation Sequencing for the Study of Flesh Color Depletion in Atlantic Salmon (Salmo Salar).” *BMC Genomics* 22 (1): 545. https://doi.org/10.1186/s12864-021-07884-9.

Voukali, Eleni, Nithya Kuttiyarthu Veetil, Pavel Němec, Pavel Stopka, and Michal Vinkler. 2021. “Comparison of Plasma and Cerebrospinal Fluid Proteomes Identifies Gene Products Guiding Adult Neurogenesis and Neural Differentiation in Birds.” *Scientific Reports* 11 (1): 5312. https://doi.org/10.1038/s41598-021-84274-x.

Voyles, Jamie, Douglas C Woodhams, Veronica Saenz, Allison Q Byrne, Rachel Perez, Gabriela Rios-Sotelo, Mason J Ryan, Molly C Bletz, Florence Ann Sobell, and Shawna McLetchie. 2018.

"Shifts in Disease Dynamics in a Tropical Amphibian Assemblage Are Not Due to Pathogen Attenuation." *Science* 359 (6383): 1517–19.

Wang, Donghai, Yun You, Pei-Chun Lin, Liquan Xue, Stephan W Morris, Hu Zeng, Renren Wen, and Xin Lin. 2007. "Bcl10 Plays a Critical Role in NF-KB Activation Induced by G Protein-Coupled Receptors." *Proceedings of the National Academy of Sciences* 104 (1): 145–50.

Wang, Zhong, Mark Gerstein, and Michael Snyder. 2009. "RNA-Seq: A Revolutionary Tool for Transcriptomics." *Nature Reviews Genetics* 10 (1): 57–63. https://doi.org/10.1038/nrg2484.

Warren, Wesley C., David F. Clayton, Hans Ellegren, Arthur P. Arnold, LaDeana W. Hillier, Axel Künstner, Steve Searle, et al. 2010. "The Genome of a Songbird." *Nature* 464 (7289): 757–62. https://doi.org/10.1038/nature08819.

Wegmann, Michèle, Beatrice Voegeli, and Heinz Richner. 2015. "Parasites Suppress Immune-Enhancing Effect of Methionine in Nestling Great Tits." *Oecologia* 177: 213–21.

White, Stephanie A, and Timothy F Wright. 2021. "Why Zebra Finches Don't Get Hypercholesterolemia." *Proceedings of the National Academy of Sciences* 118 (23): e2107021118.

Wirthlin, Morgan, Nicholas CB Lima, Rafael Lucas Muniz Guedes, André ER Soares, Luiz Gonzaga P Almeida, Nathalia P Cavaleiro, Guilherme Loss de Morais, Anderson V Chaves, Jason T Howard, and Marcus de Melo Teixeira. 2018. "Parrot Genomes and the Evolution of Heightened Longevity and Cognition." *Current Biology* 28 (24): 4001–8.

Wright, JC, Robert J Beynon, and Simon J Hubbard. 2010. "Cross Species Proteomics." *Proteome Bioinformatics*, 123–35.

Wu, Runliu. 2022. "Mitochondrial ACOD1/IRG1 in Infection and Sterile Inflammation," 11.

Wutzler, R, Katharina Foerster, and Bart Kempenaers. 2012. "MHC Class I Variation in a Natural Blue Tit Population (Cyanistes Caeruleus)." *Genetica* 140: 349–64.

Xiao, Tsan Sam. 2017. "Innate Immunity and Inflammation." *Cellular & Molecular Immunology* 14 (1): 1–3.

Yang, In Seok, and Sangwoo Kim. 2015. "Analysis of Whole Transcriptome Sequencing Data: Workflow and Software."

Young, Anna M, Elizabeth A Hobson, L Bingaman Lackey, and Timothy F Wright. 2012. "Survival on the Ark: Life-history Trends in Captive Parrots." *Animal Conservation* 15 (1): 28–43.

Zann, Richard A, Stephen R Morton, Kevin R Jones, and Nancy T Burley. 1995. "The Timing of Breeding by Zebra Finches in Relation to Rainfall in Central Australia." *Emu* 95 (3): 208–22.

Zappaterra, Mauro W, and Maria K Lehtinen. 2012. "The Cerebrospinal Fluid: Regulator of Neurogenesis, Behavior, and Beyond." *Cellular and Molecular Life Sciences* 69: 2863–78.

Zeeland, Yvonne R.A. van, Berry M. Spruit, T. Bas Rodenburg, Bernd Riedstra, Yvonne M. van Hierden, Bart Buitenhuis, S. Mechiel Korte, and Johannes T. Lumeij. 2009. "Feather Damaging Behaviour in Parrots: A Review with Consideration of Comparative Aspects." *Applied Animal Behaviour Science* 121 (2): 75–95. https://doi.org/10.1016/j.applanim.2009.09.006.

Zhang, Qu, Geoffrey E Hill, Scott V Edwards, and Niclas Backström. 2014. "A House Finch (Haemorhous Mexicanus) Spleen Transcriptome Reveals Intra- and Interspecific Patterns of Gene Expression, Alternative Splicing and Genetic Diversity in Passerines." *BMC Genomics* 15 (1): 305. https://doi.org/10.1186/1471-2164-15-305.

Zhang, Xiaofei, Arne H Smits, Gabrielle BA Van Tilburg, Huib Ovaa, Wolfgang Huber, and Michiel Vermeulen. 2018. "Proteome-Wide Identification of Ubiquitin Interactions Using UbIA-MS." *Nature Protocols* 13 (3): 530–50.

Zhang, Xiaolong, Muhammad Akhtar, Yan Chen, Ziyu Ma, Yuyun Liang, Deshi Shi, Ranran Cheng, et al. 2022. "Chicken Jejunal Microbiota Improves Growth Performance by Mitigating Intestinal Inflammation." *Microbiome* 10 (1): 107. https://doi.org/10.1186/s40168-022-01299-8.

Zhao, Chunmei, Wei Deng, and Fred H Gage. 2008. "Mechanisms and Functional Implications of Adult Neurogenesis." *Cell* 132 (4): 645–60.

Zhou, Ying, Yan Hong, and Haihua Huang. 2016. “Triptolide Attenuates Inflammatory Response in Membranous Glomerulo-Nephritis Rat via Downregulation of NF-KB Signaling Pathway.” *Kidney and Blood Pressure Research* 41 (6): 901–10.

Ziv, Yaniv, Noga Ron, Oleg Butovsky, Gennady Landa, Einav Sudai, Nadav Greenberg, Hagit Cohen, Jonathan Kipnis, and Michal Schwartz. 2006. “Immune Cells Contribute to the Maintenance of Neurogenesis and Spatial Learning Abilities in Adulthood.” *Nature Neuroscience* 9 (2): 268–75.

Appendix