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Ref: PhD thesis Martin Těšický

It is a great pleasure to review the PhD thesis of Martin Těšický, which contains 15 chapters related to diverse aspects of inflammation in wild birds. I was impressed by the breadth of topics covered in this thesis, demonstrating the ability to develop diverse skill sets and to collaboratively work at the interface of different research fields. Notably, 11 of 15 chapters are already published in international peer-reviewed scientific journals (incl. 3 first-author publications). All other chapters are currently under review. This is an impressive achievement that demonstrates a very high level of commitment and dedication.

Across chapters, the candidate uses innovative, complementary approaches and techniques from ecology, evolutionary biology, physiology, immunology, microbial ecology, analytical chemistry, -omics, and bioinformatics to address timely research questions related to the causes and consequences of inflammation in wild bird. The statistical analyses are performed to a very high standard and the chapters are written in a clear and engaging scientific writing style. Throughout the thesis the candidate's thorough knowledge and deep understanding of the wider research field, as well as general ecological and evolutionary principles, became apparent, and relevant literature was adequately referenced throughout. The findings are novel and have the potential to stimulate future research across fields. Indeed, the publications have already attracted a good number of citations, demonstrating their impact.

Summary and appraisal of chapters:

Chapter 1 - 3: These chapters aim to identify sources of inter- and intraspecific variation in gut microbiota composition across bird species and find evidence for surprisingly weak effects of geography, life history and ecology, highlighting that different factors shape within-host microbial communities in mammals and birds. Chapter 3 also makes an important methodological contribution to the analysis of microbial communities and provides guidance for future studies.

Chapters 4 & 5 test for differences in gut microbiota composition across the gastrointestinal tract of passerines and parrots, and find clear oral vs faecal differences, but little evidence for differentiation among gut locations, again contrasting findings in mammals. The results also provide evidence that faecal samples, which are often used in ecological studies, represent microbial communities of the gut well, and are superior to cloacal swaps.

Chapter 6 tests for possible vertical or horizontal trans-shell transmission of gut microbes from mother to offspring. Little evidence for such vertical or horizontal trans-shell transmission was found, highlighting the key role of postnatal factors in the colonisation of the within-host microbial community. The meticulous approach to optimise the sequencing and control for potential contamination (which has been a key problem in previous studies) is exemplary and deserves special mention.

Chapter 7 is a conceptual paper that outlines approaches for identifying 'immune genes' using genomics / transcriptomics data and makes excellent suggestions for future research in the field.

Chapter 8 uses a predictive structural bioinformatics approach to develop a pipeline that aims to identify genomic regions under (pathogen-mediated) selection. The candidate then uses the pipeline to demonstrate widespread patterns of convergence in TLRs in tits, which is an exciting finding, however the drivers of convergent evolution remain unknown at this stage. The tools developed in Chapter 8 will be extremely valuable to other researchers and have the potential for strong impact in the fields of ecoimmunology and immunogenetics.

Chapters 9-11 investigate patterns of variation and signals of selection in different bird groups and find strong evidence for selection in different classes of immune genes (incl. TLRs, RLRs). Of particular interest is the lack of evidence for trans-species polymorphisms in TLRs in tits. The work also demonstrates that MDA5 and RIG-I underwent multiple gene losses in bird evolution (similar to mammal evolution). The causes and consequences of such gene losses remain currently unknown and will be an interesting topic for discussion during the defence, and a fruitful avenue for future research.

Chapter 12 demonstrates CNR2 loss in parrots and uses in vivo experiments to show that the loss of CNR2 is associated with increased inflammation in the brain in response to an immune challenge. These are intriguing findings, and they raise the question why selection has not prevented the loss of these genes or favoured compensatory mechanisms to prevent neuroinflammation – a topic which I am looking forward to discussing in more detail during the defence.

Chapters 13 – 15 use a longitudinal approach to test for age-related changes in multiple physiological traits, including plasma testosterone levels, inflammation and heavy metal concentrations. A significant polynomic decline of circulating testosterone levels ('endocrinological senescence') and a significant linear increase in levels of chronic inflammation ('inflammaging') with age was found. It is for the first time that 'inflammaging' has been described in a wild bird population using a longitudinal approach and these findings are thus likely to have a significant impact in the field of biogerontology. Interestingly, no clear relationship between age and heavy metal concentrations (except for Pb) was found. An interesting next step would be to test how the different physiological measures are associated with age-related changes in reproductive success and survival to gain insights into the proximate mechanisms underlying the ageing process, and although very challenging, to experimentally manipulate the different physiological traits to quantify their age-specific impact on fitness components.

Overall, I thoroughly enjoyed reading this excellent thesis and wholeheartedly recommend it for defence. I declare that I have no competing interests or possible biases in relation to the candidate or the presented work.

I am looking forward to discussing the findings with the candidate in more detail during the defence.

Yours sincerely

Barbara Tschirren