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Review of PhD thesis by Balraj Melepat

The thesis is based on six papers and Melepat is the lead author on three of these. The empirical studies can be grouped into two topics. There are three papers on neuroinflammation in birds and two papers on immune responses to *Mycoplasma* infection in houses finches. The thesis also contains a review on the molecular evolution of vertebrate Pattern Recognition Receptors (PRR) involved in sensing viral infection. The topics are somewhat disparate, although innate immune responses in birds is a unifying theme.

The studies of neuroinflammation build on previous work by the same group. The new studies extend the previous work, for example by investigating the effect of immune challenge on expression of sensors like *TLR3* and signaling genes like *CASP1* in the CNS (in addition to cytokines like *IL6* and *IL1B* as in previous studies).

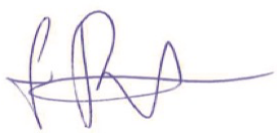
The studies of *Mycoplasma* in house finches also build on previous work by the same group, where it was shown that house finches primarily have evolved tolerance (rather than resistance) to this new pathogen. The new studies focus on investigating the immunological mechanisms behind tolerance in more detail and indicate that it is a result of attenuated expression of proinflammatory cytokines.

The studies are in general well performed. The studies on neuroinflammation are interesting but appear a bit preliminary and more work on this topic is clearly required before strong conclusions about the causes and consequences of neuroinflammation in parrots and other birds can be drawn. On the other hand, the work on cytokine expression of immune genes in houses finches during *Mycoplasma* infection is very comprehensive and is based on an impressive experiment involving birds from four different populations across North America; the results are novel and very exciting, and I expect these studies to become well cited examples of host adaptation in response to a new pathogen.

The thesis provides relevant background information and an adequate summary of the different papers. Overall, the quantity and quality of work is clearly sufficient, and I recommend the thesis for defence.

I have no conflicts of interest.

Lund 19th September 2024



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Some comments:
The title of the thesis appears to mainly cover the studies on neuroinflammation (but not the finch work).

Dividing the General Introduction and General Results and Discussion into sections (with subheadings) would enhance readability.

The outline of the Red Queen hypothesis and its relevance for the present thesis is not entirely clear (p 5).

Questions:
On several occasions (p 1, p 6), you write more or less explicitly that birds are particularly important as hosts for pathogens; is that really the case? And does it really matter for your studies?

Can you speculate on why PRRs sensing viruses are more constrained than other PRRs?

Would you expect positive selection on viral sensing PRRs to mainly target ligand-binding domains, signaling domains, or other domains? What are the empirical patterns? What kind of host-pathogen interactions could impose selection on the different types of domains?

How does NLRP3 sense viral infection?

Are there any special circumstances that make it more likely that peripheral inflammation leads to inflammation in CNS (p 4)? Type/site of immune challenge, general condition of host individual, etc?

What is the function of *CNR2* (p 7)? What could be the reason behind loss of *CNR2* in parrots? Are there other birds, or other vertebrates, that have lost this gene? Are parrots more susceptible to neuroinflammation than most other bird species, not just zebra finches? How can the role of (loss of) *CNR2* in neuroinflammation (in parrots) be confirmed?

Which other host species do *Mycoplasma gallisepticum* occur in?

How did you calculate relative expression (R), given that you don't have paired control and treated samples?

The differences in expression of *IL1B* and *BCL10* between house finches from VA and IA is intriguing and suggest (as you write in paper 6) that different house finch populations have evolved tolerance in different ways. How would you do to confirm that the changes in expression of these genes really contribute to tolerance?

If you want to find the genetic basis (i.e. SNPs where allele frequencies have changed) behind changes in expression of *IL1B, IL10* and *BCL10* in response to *Mycoplasma* between house finch populations, where would you look? In or near these genes, or elsewhere in the genome? In exons or other parts of genes?