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**Evaluation of the PhD thesis of Benita C. Laird-Hopkins**

The above-mentioned thesis addresses butterfly thermoregulation across habitats and climatic regions. I really enjoyed reading this thesis, which I found to be an excellent piece of work. So, first of all, I would like to congratulate the candidate as well as the supervisors. In times of rapidly changing climatic conditions, investigating thermal biology in a variety of species is much needed. Thermoregulation is an important aspect of thermal biology, which is what the thesis focusses on. Thus, the thesis is certainly timely, providing novel and much needed data. By investigating thermoregulation across different spatial scales, the thesis is embedded within a strong framework. It has an exceedingly clear structure and logical flow. I was also impressed by the clever experimental designs used in the different chapters, and specifically comment on considering morphology, physiology and behaviour within a comparative approach. Another important strength of the thesis is the combination of field and laboratory approaches.

**Chapter 1 - Introduction**

The thesis starts with a thoughtful introduction, which is very clear in terms of structure and content. It very nicely sets the scene for the following chapters. It emphasizes the importance of a solid understanding of the responses of organisms to temperature and humidity for predicting the impacts of climate change. The introduction also nicely highlights the gaps in our current understanding, such as the lack of studies on topical butterflies. The following three chapters address these gaps

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and are all of very high quality, which comes as no surprise as two papers are already published in highly prestigious journals, and the third one is already submitted.

**Chapter 2 - Neotropical and temperate butterflies**

In chapter 2, the thermoregulatory abilities of neotropical and temperate butterflies were investigated. To my mind, this is a very important and novel contribution to science, as much needed data on tropical (and temperate) butterflies is provided, and as measurements were taken in nature rather than in artificial laboratory environments. A large data set has been gathered, which was subsequently eminently analysed. Neotropical butterflies were better at buffering their thorax temperature at higher ambient temperatures than temperate butterflies. These differences were due to differences in wing morphology. This chapter is an important contribution towards a better understanding of the thermal biology of tropical insects, and has consequently been published in a world-leading journal.

**Chapter 3 – Thermoregulation in European butterflies**

Chapter 3 investigates thermoregulation in European butterflies, again using field data which I really think is an asset. The results show that butterflies from warmer regions have a higher buffering ability than those from cooler regions. However, within species no consistent pattern across regions was found. This chapter nicely illustrates the complexity of thermoregulation, and that behaviour and microclimate are important to consider.

**Chapter 4 – Thermoregulation in *Heliconius* butterflies**

This study is completely devoted to tropical butterflies, specifically the fascinating *Heliconius* butterflies. The results show that thermoregulation is driven by morphology rather than phylogeny. Furthermore, butterflies from cooler habitats showed a higher buffering ability and took off at lower temperatures. The study includes both field and laboratory measurements, which is in my opinion a major strength of the paper. Also, making use of the Müllerian mimics is an excellent idea to test the

hypotheses put forward, as is the comparison of dead and alive butterflies. I am really impressed by the clever design of this study, which has, again, been already published in a highly renowned journal.

**Chapter 5 – Summary**
The summary nicely wraps up the principal findings of the thesis and puts them into the relevant context.

Overall, this thesis is in my opinion a major contribution to our understanding of thermoregulation and its underlying drivers and mechanisms. In particular, it deepens our knowledge on thermoregulation in tropical butterflies substantially, for which there is the highest need for additional data. I am a bit less enthusiastic about the implications for predicting vulnerability to climate change (see below).

**As far as I am concerned the thesis can be accepted without hesitation. Thus, I suggest to accept this thesis in its current form.**

**Questions**

(1) In your introduction, you frequently use the term 'acclimation'. Later you state that a given pattern might result from acclimation or plasticity. Could you please define the term 'acclimation' and explain how it relates to 'phenotypic plasticity' and other types of thermal plasticity?

(2) Repeatedly, it is mentioned that differences in thermoregulation may be due to phenotypic plasticity or genetic adaptation. Which of the two do you think plays a larger role for the patterns you have observed, and how can this be tested (i.e. how can you differentiate between phenotypic plasticity and genetic adaptation)?

(3) Your hypotheses often rest on considerations of thermal tolerance, but you investigate thermoregulation. Could you please explain what we can learn from studying

thermoregulation (e.g. thermal buffering, heating rate, take-off temperature) for the sensitivity of organisms to climate change?

(4) Higher buffering ability was largely explained by morphology in your first study. Thus, better buffering ability is associated with larger body size. However, this does not mean that body size / wing length is the target of selection (the spandrels of San Marco…). Could you speculate about factors that might select for or plastically enlarge body size in tropical regions?

Yours sincerely,

Prof. Dr. Klaus Fischer