Report on the doctoral dissertation of Yannick Geert Klomberg: 'The role of plant functional traits in organising plant-pollinator interactions'. Charles University, Czech Republic, April 2021

This dissertation comprises an Introduction followed by six data chapters, each presented as either an unpublished manuscript or as a PDF of a published paper.

The Introduction does a very good job of setting the background to the research, discussing important aspects of the history of pollination ecology as a topic of study. An important point that is made here, and then picked up in the subsequent chapters, is that there have been relatively few studies of pollination ecology and floral evolution from the two regions that are the focus of this dissertation: the Afrotropics and Central Europe. As such the work in this dissertation considerably extends our geographical understanding of the ecology of flowers and their pollinators into regions where there is a lack of data on plant-pollinator interactions.

Not only is there a wide geographic scope to this body of work, but there's also a broad range of approaches used, from field observations through to glasshouse studies, and experiments in the field, and some very sophisticated statistical modelling. The focus of the work also shifts from interactions and a zoological perspective, to purely botanical when considering mating systems. Collaboration with colleagues and supervisors has been a key part of the work and it's clearly provided excellent training and experience for the candidate.

Another thing that I especially like about this body of work is that it is not afraid to challenges some of the traditional ideas about pollination syndromes and the role of floral traits in attracting and rewarding pollinators, and in determining the level of pollen limitation and selfing.

Overall the unpublished parts of the dissertation are well written, though there are a few places where the English grammar needs attention, and where ideas could be more accurately expressed. I have noted a few of these below.

Some of the work is already published and some has or is about to be submitted.

In the remainder of this report I will describe the main data chapters in turn and pull out some key results and questions that I think are important to consider.

Chapter I entitled ‘Spatiotemporal shifts in the role of floral traits in shaping tropical plant-pollinator interactions’ is presented as an unpublished manuscript, though published as a preprint in BioRxiv.

This study tests the ‘pollination syndrome hypothesis’ by using data on floral traits and pollinators along an elevational gradient on Mount Cameroon during two seasons. It uses a relatively novel application of Random Forest models, and finds that some traits have more predictive utility than others, but that this varies according to environment and pollination system.

In the Introduction to this chapter, I think that perhaps the idea that some studies ‘support’ and other studies ‘contradict’ the ‘validity’ of the hypothesis, as set out in the Introduction, is an unnecessarily polarised view of the debate. Even Ollerton et al. (2009) ‘supports’ the hypothesis, albeit for only 30% of the plant species. Otherwise the Introduction frames the debates around syndromes very well. I especially like Figure 1 in this chapter as visually conceptualising some of the issues.

Chapter II is entitled ‘Elevational and seasonal patterns of butterflies and hawkmoths in plant-pollinator networks in tropical rainforests of Mount Cameroon’, and is an unpublished manuscript with Mertens as first author.

The study looks at patterns of interactions with flowers, and how this changes with elevation, in two groups of Lepidoptera that are known to be important pollinators in some other tropical plant communities. There is a very strong elevational pattern, with the number of species declining dramatically as one progresses up the mountain. However overall it is surprising to me how infrequent these groups are as pollinators, compared to other studies. That’s certainly something which I would like to discuss during the examination – why is Mount Cameroon so different in this regard?

One issue that I did spot is that on p4 of the chapter it’s stated that: ‘Individual lepidopteran groups differ in their morphological and behavioural adaptations to pollination.’ With the exception of a very small number of ‘active’ pollinators (some fig wasps and yucca moths for instance) pollinators are never ‘adapted to pollination’. They are adapted for flower visitation, but pollination is a side-effect of that visitation.

Chapter III is a submitted manuscript with Chmel as first author, entitled 'Bird pollination syndrome works as the plant's adaptation to ornithophily, while nectarivorous birds do not seem to care'

In this study the authors have looked at the relationship between floral traits of plant species and flower visitation by sunbirds. They find a strongly asymmetric relationship, with flowers specifically adapted to sunbird pollination, but the birds themselves visiting a wide range of different flower types. This mirrors what has been found in hummingbirds and is a very nice confirmation of a general pattern in bird exploitation of flowers.

The conceptual model presented in Figure 1 is very useful, though it does exclude the role of other vertebrates, e.g. plants that are pollinated by both birds and bats.

On p9 of the manuscript there's an odd statement that I didn't understand: 'we considered flowers of plants to fall within the bird pollination syndrome if they: …. 5) had tubes or spurs too narrow for nectarivorous birds'. Is this an error?

In relation to the methods, can we always be sure that a bird feeding on a flower is actually feeding on the nectar and not on insects in the flower? And does it matter from the flower's perspective?

Interesting that only 9 of the 48 plant species (<20%) had traits associated with traditional bird pollination syndrome.

You should change the colour coding in Figure 3 as people who are red-green colour blind will not be able to distinguish the data points.

P18 & Table 1 – 'Bird visitation frequency tended to be higher on flowers: that exuded odour' – were you surprised by that? Not a great deal is made of this finding in the Discussion. However, there is a very low R^2 to that correlation.

In **Chapter IV**, in a paper published in 2020 in *Plant Biology* (Bartoš as first author) entitled 'Self-compatibility and autonomous selfing of plants in meadow communities.', the focus shifts from African to Czech vegetation. There is also a change of emphasis from thinking about species interactions within a community to assessing plant reproduction very much from a plant-only perspective. It will be interesting to discuss how comfortable candidate is

with this shift in emphasis. It requires a rather different skill set and deals with a very different literature, in comparison to the species interactions studies.

From Fig. 1 – why do the SI non-selfers produce so little nectar in comparison to the SC non-selfers? That seems counter-instuitive to me.

In **Chapter V** we stay with Czech plant communities for a study published in the journal *Plants*, again in 2020, entitled 'Are reproductive traits related to pollen limitation in plants? A case study from a Central European meadow', with Bartoš as first author.

This study deals with an important but still poorly-understood topic in pollination ecology: the extent and drivers of pollen-limitation of plant reproduction. The main finding, that pollen limitation decreased when more functional groups of pollinators was involved, is a fascinating one. It has significant implications for the evolution of more generalised pollination systems in flowering plants. However, Fig 2B suggests a more complex scenario in which pollen limitation increases when there are a high number of functional groups (>5). Why might that be? Some suggestions are made in the paper but I'd like to discuss this in more detail.

The final **Chapter VI** is a paper entitled 'The role of ultraviolet reflectance and pattern in the pollination system of *Hypoxis camerooniana* (Hypoxidaceae)' and published in AoB PLANTS in 2019.

In this research, which takes us back to Mt Cameroon, the authors demonstrated the importance of ultraviolet (UV) reflection for pollination of these flowers by bees. They manipulated the flowers by applying a 'sunscreen' that prevented UV reflectance. It's a very neat experiment, following on from previous work by Steve Johnson's group. I was surprised to see that they felt it necessary to comment on using a natural, unmanipulated control as that previous study had not done so. That surprised me – I would have thought it would be the obvious thing to do!

In conclusion, I am happy to state that the dissertation presented by Yannick Klomberg can be recommended to be accepted by the Faculty of Science of the Charles University in Prague, that I am of the opinion that the dissertation should be deemed to have passed, and should go forward to the defence stage.

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