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Review

of the doctoral thesis of Yannick Geert Klomberg MSc

'The role of plant functional traits in organizing plant-pollinator interactions'

Yannick Klomberg presents a doctoral thesis with six chapters, each representing one publication. All publications have multiple authors. Three manuscripts have not yet been published, three manuscript have been published in international peer-reviewed botanical journals with impact factors higher than 2. Yannick Klomberg is the first authors of two publications and significantly contributed to all publications and manuscripts at least by means of field work, data processing and manuscript editing.

The overall goal of the dissertation is the re-evaluation of functional floral traits and its predictive power for plant-pollinator interactions. Yannick Klomberg's use of various descriptive, experimental and theoretical approaches to solve this goal is impressive.

Yannick Klomberg begins with an overview about history and perspectives of pollination research, interactions of pollinators with flowers and their specialisations, definitions of floral functional traits, annotation of the pollination syndrome hypothesis, introduction of the study sites at Mount Cameroon and meadows of the Železné Hory Protected Landscape area, and explanation of aims and outcomes of the research projects.

One main study is explicitly about of the predictive power of pollination syndromes. The study took place at an elevational gradient of tropical rainforest at Mount Cameroon. This study found that the importance of distinct floral traits to predict the pollinating group of animals differs and varies with environmental conditions. Yannick Klomberg presents a profound understanding of the concept of pollination syndromes and the critique about the validity of this concept. Yannick Klomberg is aware that the conceptualization of the pollination syndromes hypothesis, as he terms it, dates back to periods of descriptive

pollination ecology and was developed by Frederico Delpino and mainly by Stefan Vogel. Yannick Klomberg knows the development of the concept of pollination syndromes over centuries and tries to translate it into the recent period of hypothesis-driven experimental approaches to research questions in pollination biology. Using Random Forest modelling the study shows that following a predetermined list of equally weighted traits can be problematic for classifying potential pollinators on the basis of the pollination syndromes concept.

The study about elevational and seasonal patterns of butterflies and hawkmoths in plant-pollinator networks of tropical rainforests at Mount Cameroon also focused on floral traits in the framework of pollination syndromes. The visits of butterflies and sphingid moths were filmed and thus based on more reliable data than pure observations. Redundancy analyses revealed significant preferences of butterflies from several families and sphingid moths to floral traits. Based on nectar production flower size and flower colour the study could separate the preferences of the butterflies and sphingid moths as predicted by the concept of pollination syndromes.

The study of the bird pollination syndrome is very inventive. The study is based on floral traits including minimum floral size, minimum tube length, absence of odour, red or orange flower colour, absence of nectar guides, and minimum nectar sugar production, placement of pollen during bird visits, which have been reported to constitute the bird pollination syndrome. These traits effectively predicted bird-pollinated flowers; however, birds also visited flowers that did not show the bird pollination syndrome. In this study functional story telling meets modern statistical approaches. The study focuses on the visitor perspective, i.e. what defines the attractiveness of a flower and consequently the floral choice of the birds, and finds that the flowers possess floral traits documenting the adaptation to pollination by birds, but the birds' choice of flowers is mainly driven by nectar availability. Interestingly, the red colour of many bird-pollinated flowers does not predict bird visitation frequency. The study does not consider the literature data about colour preferences in flower-visiting birds.

Two publications deal with a wet meadow system in Europe and investigate self-compatibility and autonomous selfing of plants as well as pollen limitation. These studies are deviant from the previous publications, but nevertheless flower traits are analysed. The descriptive and experimental approach listed the frequency of fully self-incompatible, partly self-incompatible species, self-compatible non-selfers and mixed mating species and constitutes the absence of inbreeding depression in most species. Floral traits, such as the level of dichogamy and amount of nectar, strongly affected the balance between selfing and outcrossing rates in the self-compatible species. Pollen limitation was not related to floral traits such as level of dichogamy and amount of nectar reward.

The publication about the pollinators of *Hypoxis camerooniana* on a montane grassland of Mount Cameroon is based on an experimental setting and demonstrates alteration of flower-visitors' response to flowers, in which all petals or half of the petals were covered with a UV-absorbing cream (decreasing the UV reflection of the petals) and appropriate controls. The flower visitors' orientation at the flowers was recorded by cameras and included landings, place of landing and movement on flowers. This study considers colour vision models of bees

and flies and thus to treats UV-reflection properties as a contribution to the perceived colour. A weakness of this kind of experiments is the unknown experience of the observed flower visitors. Most likely nearly all of the observed flower visitors had visited already natural flowers of *H. camerooniana* and thus were searching for similarly looking flowers. In this regard the authors simply show colour learning and colour discrimination in bees. If bees are trained or experienced to a distinct colour they will negatively respond to any alteration of the trained stimulus; however, innate preference may still work even in experienced flies and bees.

Yannick Klomberg combines challenging fieldwork such as in the wet season in the tropical rainforest of Mount Cameroon with novel approached to classical and long standing hypotheses. The overall introduction and the publications document a provocative writing style and a profound knowledge of the elder and recent literature about flower traits. The statistical analyses and diagrams are meaningful and easy to understand for readers. All studies presented in the dissertation keep the focus on functional floral traits and its predictive power for pollinators. The main outcome of Yannick Klomberg's studies is that the predictive ability of floral traits differed spatiotemporally and among pollinator functional groups.

All in all Yannick Klomberg presented a cumulative doctoral thesis, which is very good in terms of comprehensiveness and quality. To the Faculty of Science of the Charles University in Prague I recommend to accept the thesis of Yannick Klomberg, and I award this work the score of "passed"

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Prof. Dr. Klaus Lunau

Questionnaire

The thin line between the acknowledgement of Stefan Vogel`s pioneering work on pollination syndromes and the rejection of the pollination syndrome concept is interesting. Is it conceivable that Stefan Vogel ever intended a statistical approach to pollination syndromes?

The butterflies' and hawkmoths' preferences for distinct floral traits is deduced from the visitation frequencies of the species studied, but ignores that the butterfly and hawkmoth species might exhibit distinct innate preferences for floral traits that might change due to negative experience with flowers presenting these traits. Particularly, colour preferences might be important for naïve animals to initially find flowers, but later are overridden by experience. Does the concept of floral traits determining pollinator behaviour consider innate preferences of pollinators?

The red colour of many bird-pollinated flowers does not predict bird visitation frequency. Is it thus possible that the red colour is not a signal to attract birds, but a signal to deter nectar- and pollen robbing bees? Irrespective of the answer, is it relevant for the concept of pollination syndromes?

Self-compatibility, outcrossing, and pollen limitation are major features in mating systems of flowering plants. Why are increased nectar volume or sucrose reward, besides dichogamy, the sole floral traits that positively correlate with the major features in mating systems of flowering plants? Is there a correlation between nectar production of bagged flowers and nectar standing crop of freely accessible flower? Is female choice a neglected feature of mating systems of flowering plants?

How relevant is the choice behaviour between flowers with manipulated UV-reflection and unmanipulated flowers, given the fact that the tested flower visitors have previously experienced only unmanipulated flowers and thus preferentially visit unmanipulated flowers due to flower constancy?