University of South Bohemia

Faculty of Science

Review of a Ph.D. thesis entitled 'Plant-Animal Interactions in an Inselberg Ecosystem: The effect on Reproduction of Selected species' presented by an applicant Blanka Vlasáková

Referee: RNDr. Jana Jersáková, PhD.

The submitted thesis consists of three manuscripts and one published paper dealing with pollination and recruitment of selected plants growing in a specific tropical ecosystem of rock outcrops in French Guiana. The thesis focused on three representatives of dominant plant communities: shrub patches dominated by two species of genus *Clusia* and monocotyledonous mats dominated by a bromeliad *Pitcairnia geyskesii*. The aim of the thesis was to describe pollination systems, role of rewards in pollination and properties of seed recruitment in *Clusia* species and an influence of pollen robbing on reproductive success of a bromeliad species. The ms. entitled "Cockroaches as pollinators of *Clusia* aff. *selllowiana* (Clusiaceae) on Inselbergs in French Guyana" was published in 2008 in Annals of Botany.

My general impression from the submitted thesis is that all four ms. are largely descriptive with several manipulative experiments which however do not often fully answer the hypotheses and leave some room for doubts and speculations. The collected data are certainly valuable (I think nobody can dispute the difficulty to reach an inselberg and determination of the applicant to work in such harsh environment) and they are surely publishable in international journals, such as Plant Systematic and Evolution, Plants Biology etc., but the main drawback I see is that they represent case studies of individual species at a particular site and that limits significance of the results for the generalization and an overall contribution to the field of pollination and reproductive biology. There are already several papers published on ecology and pollination of *Clusia* species and I would appreciate if the applicant could briefly summarize what makes her research different from the other published studies and novel within an international context.

When I started to read individual ms. I was very excited by the topic of the first two ms. dealing with unusual pollination system, unusual reward and a potential existence of automimetism. Unfortunately, I consider both of them to be not well designed, with several methodological problems, and with interesting but not fully supported results. For example the existence of automimicry in *Clusia* and the role of acetoin in cockroach attraction were

not properly tested. I believe that these problems stem mainly from the fact that though the topics largely dealt with pollination biology the project designs were not co-supervised or consulted with a specialist in pollination biology field. The third ms. dealing with effects of seed size and microhabitat on recruitment of two *Clusia* species is better designed, well written and discussed and for me represents the best ms. of this thesis. The last ms. on temporal changes in pollen-robbing in a bromeliad species has a character of a short communication paper with rather short discussion, which is not addressing well the significance of observed temporal variation; also the issue of pollen robbing is not discussed in broader context. It is well known that intensity of reward robbing and pollination rate might differ among the years and thus predictions of selection on floral traits should not be based on a single season. The detailed questions and major remarks to individual ms. are listed bellow.

From the formal point of view, the thesis is well organized and has a standard structure. In some cases I would like to see more post-hoc statistical tests testing differences between individual treatments and consistent report of the number of samples used (see minor remarks listed bellow).

To conclude, my major criticism refers to narrowness of the selected topics. I think that the selected systems offered some really nice broader topics which could have been explored, for example the evolution of floral rewards in *Clusia* genus, evolution of cockroach pollination, effect of seed size and capsule type on distribution of *Clusia* genus in tropics etc. On the other hand, I understand that such broad topics might need larger scientific team and more than two seasons for data collection. My second question is why the applicant worked on a particular inselberg, was it the only one which was accessible? When she used a helicopter was not it possible to visit also some other places to verify the results obtained at Nouragues inselberg?

The extent of the data and overall quality of the ms. are sufficient to fulfil demands for a Ph.D. thesis. The applicant demonstrated ability to work independently, set objectives, analyze data and discuss them in more or less broader context. Therefore I recommend this thesis to be accepted for the award of PhD.

Major remarks and questions:

Paper 1:

1. Flower visitors: I would appreciate to have a table with flower visitors and their visitation rate. Would the author know some other pollination systems on islands, which also involve omnivorous insect feeding on nectar during night?

2. Pollen load on cockroaches: the applicant found numerous pollen grains on cockroach's bodies, but lower amount of pollen when she washed bodies with ethanol. I am missing information how many grains were found directly on the bodies compared to body washing? Is ethanol a good substance for this purpose? Which other reagents would be more suitable? Does she know some other methods how to determine pollen load on insect bodies?

3. Scent composition: Was the scent composition same for male and female flowers? If not could the differences explain different visitation rates to the sexes?

4. Presence of sugars in secretion: Are there some other methods how to prove presence of sugars in liquids directly in the field then by HPLC? Was the amount of secretion same in both sexes and what was the volume? If not the same, could it explain differences in visitation rate between male and female flowers?

5. Field bioassay: There are several methodological problems and I think the author is aware of some of them, because she has the following text in her discussion: "The relatively low numbers of cockroaches that were observed visiting a model might also be the result of non-optimal concentrations. In many Lepidopteran species a high concentration of sex pheromones repels rather than attract insect. Furthermore acetoin may not be the only compound that attracts cockroaches to the flowers." Why was not the concentration suitably adjusted and which protocol is normally used for this purpose? Why was the physiologically active response of cockroaches measured only to acetoin and not to all compounds found in the scent blend? What can be concluded from the visitation of flower models and vials with acetoin, when there is no control? How this control should be designed? How the different doses of acetoin used in EAD correlate with the amount of acetoin in real flowers?

6. Floral secretion: In discussion the author suggested that the staining of floral secretion with Neutral red could indicate presence of non-volatile oils. To which compounds Neutral Red has an affinity? Why is this stain used to locate osmophores? Can the author review pollination systems using oil as a reward? Does such system exist also in Europe? Why the floral secretion was not subjected to GS-MS? Could be the oil presence related to cockroach's nutrition?

7. Presence of acetoin in cockroaches: Which method could be used to detect presence of acetoin on cockroach's body?

Paper 2 :

1. Title: I think the title is a bit misleading, as the paper deals only with female function (i.e. capsule production), does not determine pollen transport and besides automimetism and floral resin the system involves also pollen reward. Therefore also the question in the last paragraph of the ms. "Which floral traits take major part in pollen transfer" could not be answered by this study.
2. Terminology: mistake pollination is less used term than deceit pollination, automimetism less frequent than automimicry.
3. Pollinator preferences and automimicry: The observation of visitation rate of male and female flowers does no give an answer to the question, if the pollinators distinguish between male and female flowers which is the most important step in determining floral mimicry. Therefore the sentence in a discussion that "bees did not distinguish between the different flower sexes" is not correct. Offering different types of flowers to pollinators on a choice stick is a typical method to test pollinator preferences and existence of mimicry. The author reported that 55 visits at female flowers were not associated with any activity. Were such visits observed also at male flowers? Is the presence of insect in vicinity of a flower a good predictor for mistake pollination?
4. Pollen transfer: As the bees can be temporarily specialized either for pollen or resin collection and thus visit just one sex, it would have been valuable to determine if pollen collecting bees visit also female flowers and vice versa. This could be achieved by marking individual bees and using capture-recapture method.
5. Tables: Table 1 could be combined with Table 2 and present the number of visitors recorded on both site, the number of total contacts etc.

Paper 3:

1. Seed handling: The author stated in the discussion that "seeds that accidentally fall on the ground have similar probability of successful germination as those that have been handled by birds." On which grounds was this assumption made?
2. Microhabitats: How many repetitions of the microhabitats types were chosen for seed sowing?
3. Transects: What is the advantage of the transect method compared to the random selection of individual habitat types in sufficient repetitions with counting the numbers of seedling in a plot?

4. Mycorrhiza: Could mycorrhiza play some role in the establishment or survival of *Clusia* seedlings?

Paper 4:

1. Flower morphology: I am missing more detailed description of the morphology of the *Pitcairnia* flower to elucidate to readers, why pollen robbers rob the pollen through the corolla and not from the front of a flower. Where are the anthers located? In hummingbird-pollinated flowers the anthers are usually extended out of the flowers as bird beak is not a good place to lay pollen. Was it possible to distinguish between pollen and nectar robbing?
2. Can be the results of this study generalized also for other *Pitcairnia* populations? Do the species of robbing bees occur also in other *Pitcairnia* populations?
3. Reproductive system: On how many plants were the manipulated flowers selected? Were the undamaged control flowers protected against damage which could happen afterwards? What was the main cause of no seed production in damaged flowers? Does the damaged flower deter the hummingbirds?
4. Temporary rate of damaged flowers: The differences between intervals were significantly different, but would be intervals 1-5 different from each other if post-hoc comparisons were applied? Therefore the hypothesis about selection towards early or perhaps simultaneous flowering is just a speculation. To state that there is indeed selection it would be useful to record not only damaged and undamaged flowers but also the final fruit set in those particular intervals. It might happen that less damage in early flowering season is counterbalanced by higher seed predation etc.
5. Pollination efficiency of hummingbirds: How was the value of 19%, mentioned in the second sentence of the discussion, determined? In the results the average seed set of an inflorescence is reported to be 3.4%. I am missing discussion of the obtained results with the paper on nectar robbery in *Pitcairnia angustifolia* by hummingbirds (Fumero-Caban & Melendez-Ackerman 2007).
6. Pollen and nectar robbing: Is the impact of nectar or pollen robbing on reproductive success in general influenced by pollen limitation? Which other pollinator groups beside *Trigona* bees are generally involved either in nectar or pollen robbing? Which other plant families are involved in pollen robbing by *Trigona* bees? Can the author imagine a case were nectar robbing could enhance reproductive success of the whole

plant population in case the robber steals nectar through the hole in a corolla and does not transfer any pollen?

Minor remarks:

Paper 1:

- Fig. 3. – Percentage of wilted flowers is cumulative, so it should be clearly stated in figure caption.

Paper 2: The citations in text should be organized alphabetically or more preferably by year in ascending manner.

- page 45: Redundant „2“ in the last sentence of first paragraph

- Fig. 1. – Percentage of wilted flowers is cumulative, so it should be clearly stated in figure caption.

- page 53: The numbers of contacts at inselberg site for male and female flowers slightly differ from those in table 2 (e.g. 32.14 vs. 32.32).

Paper 3:

- page 67: Missing the number of capsules dissected to estimate seed set.

- Fig. 2 and 3: Missing error bars.

Paper 4:

- Abstract: redundant word “attacked” in text “Attacked flowers attacked...”

- page 84: The number of degrees in the test showing differences in seed set caused by the pollen origin is reported to be 6. But there were only 3 pollination treatments.

- page 85: The average seed set of survived flowers – is it equal to capsule set? I suggest distinguishing between seed set and capsule set.

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