Review of the PhD thesis submitted by Michael Mikat

I very much enjoyed reading this thesis. It is an exceedingly excellent investigation of social evolution using *Ceratina* bees as a model organism. The majority of chapters have already been published and therefore have gone through rigorous reviewing processes, and the candidate must be congratulated for this high level of reviewed output, it is very impressive!

I provide a range of comments on the thesis below. The thesis is much larger than is expected for a PhD. Although it contains 8 separate 'papers' (though Study 7 appears to be missing), a smaller number of these would be more than adequate for a PhD.

I was very impressed with the breadth of the chapters and with how the candidate was able to identify, and address, so many issues associated with social evolution.

My comments on the thesis are given below. *These comments provide various suggestions and my responses to some of the material in the thesis, but I do not require the candidate to formally address these, they are suggestions for thinking about*. **However, I outline two questions at the end of this assessment that I would like the candidate to address in the viva.**

Prior to the viva, my recommendation would be that the thesis be accepted with minimal changes and those changes only need to be approved by the candidate's supervisors. The thesis does not require re-examination by myself.

This is a truly outstanding thesis. Mikat has already had a major impact in the field of insect social evolution.

Specific comments as follows:

Page 2 of thesis: You refer to small carpenter bees as genus *Ceratina*, but I would be curious to hear of you think this entire tribe should be regarded as a single genus. What do you think comprises a genus and is this different from a tribe?

Page 2: you say that most of the studied species are eusocial, yet I know that many people would disagree. I would like to hear what you understand by the term "eusocial".

Introduction.

The introductory chapter is very comprehensive and exceedingly well researched. This chapter could easily be reconfigured as a very solid review paper and I would encourage the candidate to do so. There are some minor grammatical issues, but these are minor and understandable for someone whose first language is not English. Overall, the introduction is very impressive in scope and understanding of multiple issues, many of which are complex. This is especially evident in the enormous number and range of the literature cited.

Some minor comments on introduction follow…

Page 6, first paragraph: you say that Hymenoptera are "extreme K-strategists", but surely that only refers to a very small proportion of Hymenoptera?! There are a very large number of Hymenoptera that are parasites and parasitoids – would you regard them as "K-strategists"?

Page 7: The first paragraph could be clearer if membership of *Ceratina* and other genera to specific tribes was made clearer, but this is a minor issue.

Page 7: there are a variety of spelling and grammatical errors here, but these are understandable given the candidate does not have English as a first language. I applaud the background provided in this section – it is a very nice summary!

Page 8 and elsewhere: The candidate frequently uses the term "nest" when the intended meaning is "colony". This is a common mistake in many publications. The "nest" refers to the physical structure, whereas "colony refers to the individuals that live within it.

Page 17: Although it is claimed that this thesis and associated papers provide the first evidence of biparental care in bees, Kukuk & Schwarz reported likely guarding behaviour in males of a communal *Lasioglossum* species (Pan-Pacific Entomologist 1988). Interestingly, we have recently found males in some *Exoneura* species will provide food to their adult female relatives via trophallaxis, though this has not been published yet.

Page 19: Although I understand why some gall-forming thrips are reported here as being eusocial, it is worth noting that "solders" in these thrips are nearly always reproductive (males and females) and in at least one species, "soldiers" have larger ovaries than their foundress mothers. The notion of eusociality in thrips therefore needs careful evaluation.

Study 1

This chapter is already published in an excellent journal and has been through a thorough review process, so my comments will be moderated by this.

This chapter provides the best study of the benefits of nest guarding behaviour in any *Ceratina* reseach to date. Quantifying the benefits of guarding is difficult and few studies have attempted to do so in most social Hymenoptera.

The sheer data size used in this chapter is impressive (see Table 1) and the most interesting results (Figures 3 and 4) are clear cut. I am not aware of any other studies examining the benefits of social nesting in xylocopine bees that are more comprehensive than this paper.

Study 2

As with Study 1, this chapter has already been published after critical journal refereeing, so I will not provide detailed comments, other than to say that the candidate is to be congratulated on publishing papers in very solid journals (like this chapter, published in BES). Although Prof. Rehan was the corresponding author for this paper and designed this

project, it is clear that the candidate took major responsibility for analysing the data and writing the manuscript, so I think this forms a valid chapter for inclusion in a PhD thesis.

I think the chapter/paper makes a very strong case that first-produced 'dwarf' daughters constitute an insurance investment. As with Study 1, the data here is voluminous and compelling.

The notion that mothers may exercise parental manipulation to encourage altruism in some of their daughters is not a recent one, but this is the best study I am aware of to back up that notion with solid data. If I were to query the Discussion, I would ask for some speculation as to why parental manipulation has not lead to true worker castes in *Ceratina*, given the very long history of social living in this tribe (probably going back to the late Cretaceous).

Study 3

I was alerted to this ground-breaking paper as soon as it was published. As with Studies 1 and 2, this chapter has already been through a very rigorous review process and PNAS is one of the toughest journals to publish in. The candidate has to be congratulated on such a major achievement.

This paper has two major implications: firstly that male altruism can be clearly demonstrated in a hymenopteran species, and secondly that polyandry can still lead to complex forms of parental investment. The implications of the data are well explored and yet necessarily cautious.

I have one minor point to raise. Biparental care by both male and female 'soldiers' is well reported in studies of gall-forming social thrips. It would have been nice to see this additional example of biparental care raised in the discussion…. What are the commonalities, and differences, between this *Ceratina* species and social thrips? (and *Trypoxylan* wasps). Further exploration of these similarities and differences may well yield very broad-ranging insights.

Study 4

This is a minor letter in PNAS representing a reply to another letter (Portman) which raised some queries about the paper in Study 3. This reply letter is measured in its response and points out that Portman did not provide convincing arguments to refute the conclusions in Study 3.

Responding to comments on published articles is an important aspect of scientific publication. So although this Study 45 is very minor, it is an example of how to constructively engage in published scientific debate and I think it forms a valid chapter of this thesis.

Study 5

As with the previous Study chapters, this paper has already been through a thorough review process and is now published in a very solid journal (Ecological Entomology). So again, my comments here will be brief.

One very interesting question to arise out of this study is why males are larger than females, given that relative male:female adult size is a maternal strategy. The paper does not provide an answer to this question, though it is an important one. But addressing that question is very difficult for multiple reasons…. Male body size might confer a mating advantage, but measuring mating success is fiendishly difficult. Observing mating episodes might be one way to measure mating success, but that would not allow other factors, such as sperm competition, to be taken account of. My guess is that answering this kind of issue would require genomic approaches to paternity but they would require studies on mating and nesting behaviour of wild populations. At present, such studies are almost impossible, but the increasing ease and low cost of SNPs and other genetic markers might make addressing this issue more feasible in the future.

My overall feeling is that this chapter/paper does not convincingly answer any particular questions, but it raises important questions that most other researchers have not recognized.

Study 6

This study has been submitted to the journal Insectes Sociaux. Its main conclusion is that colony membership cannot be explained by kin selection alone. If this contention can be clearly proved, then this paper will become an important part of mounting evidence that insect sociality can be explained by non KS models, such as mutualism or other models such as reproductive queueing when resources are constrained.

I have one issue to pick with the candidate, namely lines 299-302. The references here to definitions of eusociality are very old and very few people would now regard them as being adequate. There are multiple recent studies that explore definitions of eusociality and those papers might form a better basis for contextualizing sociality in *Ceratina chalybea* here. Querying definitions of social 'levels' may appear to be pedantic, but they can help clarify the evolutionary questions that are being asked when we talk about social evolution. Dew et al. (2016, Insectes Sociaux) explore some of those issues and it would have been good to see some discussion of how definitions of eusociality can influence the interpretation of empirical studies.

Overall, I agree with the conclusion in this chapter that evolutionary models such as mutualism require more attention on social insect studies than has been provided in the past. This chapter provides good empirical support for that push.

Study 7

There does not seem to be any 'Study 7' inter-leaved between Studies 6 and 8 in the pdf thesis version I have. The final version of the thesis will need to adjust study numbers so they are continuous.

Study 8

I personally found this chapter to be one of the most interesting chapters in the thesis. In particular, longevity of females, *and especially males*, is very important for sex allocation models. Partial bivoltinism and its effects on sex allocation formed an active field of enquiry when the 'haplodiploidy hypothesis' was first being proposed by Hamilton and Maynard-Smith, but questioned by people like Trivers and Crozier. There were then some key studies by researchers like Grafen that further explored this issue.

My feeling is that this chapter is strong in terms of empirical data, but could have gone further into exploring, even in non-mathematical terms, the potential consequences of overlapping generations. Nevertheless, this is a strong chapter.

Questions that I would like to propose for the viva:

1. The Ceratinini appears to be a very ancient lineage, perhaps even back to the early Eocene, maybe earlier. Your research has uncovered some very complex behaviours in this tribe, so I am curious to hear why you think this tribe has never evolved the advanced kinds of eusocial behaviour that we find in corbiculate and halictine bees.
2. Your research has demonstrated an important role for males in the colony life of some *Ceratina* species. Do you think this has influenced sex allocation in *Ceratina*? Is there any evidence that sex allocation in *Ceratina* ever deviates from equal investment in sons and daughters?

Michael Schwarz

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