Dear Dr Niels Verhulst,

Thank you for reviewing our manuscript. We greatly appreciate the feedback from the reviewers. We have improved our manuscript based on their recommendations, and we hope that you will find the current version to be acceptable for publication. We have replied to the comments below.

We look forward to hearing from you.

Sincerely,

## Editor

Small revision in discussion required

## Dear authors

The two reviewers are positive about the revisions that were made. I agree with the second reviewer that it would be good to describe the possible limitations in a bit more detail. It is surprising that no volatiles were detected from a group of mosquitoes when compared to an empty cage and different methods may change this.

Kind regards.

We agree that changes in some parameters in our setup or different methods may provide different results. The matter of airflow is now mentioned in the discussion as requested by the reviewer 2.

However, we believe that absence of long-range volatile organic compounds or our inability to detect minute quantities of some compounds, although not expected, are maybe not as rare as it look likes. Indeed, as it is always difficult to demonstrate the absence of something, such results are probably not published and remain unnoticed. In our case, we do not affirm that no volatiles are emitted but that no long-range volatile compounds were found. As we were searching for chemicals able to attract females from distance over several meters, these long-range volatile compounds were the focus of this study. Nevertheless, low-volatility organic compounds or even non-volatile compounds such as heptacosane (Wang et al., 2021) can be

involved in the mating process at shorter ranges. This is now specified lines 543-546 and 552-554 in the new version of the manuscript.

#### <u>Reference</u>

Wang G, Diabaté A, Liu J, et al (2021) Clock genes and environmental cues coordinate *Anopheles* pheromone synthesis, swarming, and mating. Science (6527) 371:411–415. https://doi.org/10.1126/science.abd4359

# **Reviewer 1**

I am satisfied with the answers given by the authors and their revised version. I have no additional comments or suggestions.

We thank the reviewer for the time he spent in reviewing our manuscript.

**Reviewer 2:** Amendments for the revised version are highlighted in yellow in the revised version of the manuscript.

**Comment #1**: I appreciate the effort of the authors in improving the readability of the manuscript. The results of the behavioural assays are a good contribution for the literature and the authors acknowledge the limitations of the study considering that a higher number of swarming males could be needed in the experiments, and that this perhaps explains why the authors do not detect differences in volatiles compounds when comparing an empty box with a box containing the swarming males. I think the fact that the authors used quite a high flow could be an alternative explanation for the fact that they don't detect any differences between an empty box and a box containing the swarming males. It is unlikely that the insects do not emit any volatiles, even when these are not exploited by females as pheromones. Moreover, employing synthetic air or pumping air from a compressed air line with the use of additional (charcoal) filters could lead to cleaner sample collection than pumping air from the environment. I think the manuscript could still benefit from a more open discussion on these aspects, and the overall conclusion should be toned down.

Flow rates and air quality are now mentioned as potential limitations of our study (lines 543-546 in the revised version of the manuscript). Also, we deleted the last section which was too affirmative. Detailed comments:

**Comment #2**: Line 220, 236 and elsewhere: it should read dynamic headspace collection *Done*.

Comment #3: Line 221, 390: the term extraction is incorrect

The term "extraction" was replaced with "collection" throughout the text when referring to VOC "extraction" from the headspaces or from the natural swarms.

We thank the reviewer for the time he spent in reviewing our manuscript.

# End of comments